An investigation of the role of hippocampal NMDA receptors in spatial learning

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April 2006

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Doctor of Philosophy

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Your file Votre référence
ISBN: 978-0-494-27849-9
Our file Notre référence
ISBN: 978-0-494-27849-9

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If we lose our memory, we lose ourselves. Forgetting is one of the symptoms of death. Without memory, we cease to be human beings.

-Ivan Klíma

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ABSTRACT

Declarative learning entails the internalization of facts and events. This type of learning depends on the integrity of the hippocampal system. In rodents, spatial learning is studied as a model of declarative learning. In this thesis, electrophysiological and behavioral experiments assessed the role of NMDA receptors in synaptic plasticity and rats' spatial learning and memory. Primed burst potentiation (PBP), a form of synaptic strengthening, was studied in freely-behaving rats treated with NMDA receptor antagonists. The impairments caused by the antagonists correlated with those observed in behavioral studies. The results support the idea that NMDA receptors in the hippocampal system mediate the internalization of the contents and organization of new environmental information, and show that the receptors are not relevant for spatial working memory or performance once a representation of the environment is stable. The results also suggest that stable spatial representations resemble multiple relations of events and do not correspond to topographical maps of an environment. As learning proceeds, representations are activated by smaller subsets of environmental cues, which eventually become sufficient for effective navigation. The representations thus are encoded as relationships of stimuli that share similarities or that are unique to a particular event. The organization of novel information is given through NMDA receptor-mediated synaptic plasticity. This plasticity mechanism could resemble a process similar to the synaptic changes observed during PBP.

RÉSUMÉ

L'apprentissage déclaratif entraîne l'internalisation de l'information sémantique et des événements. Ce type d'apprentissage dépend de l'intégrité du système hypocampique. Chez les rongeurs, l'apprentissage spatial est étudié comme un modèle d'apprentissage déclaratif. Afin d'élaborer cette thèse, une série d'expériences comportementales et électrophysiologiques ont été réalisées ayant pour objectif l'évaluation du rôle des récepteurs NMDA dans la plasticité synaptique et dans l'apprentissage spatial chez des rats. Une forme de renforcement synaptique entraînant potentiation au moyen d'un pulse initial suivi d'une rafale de stimulation à haute fréquence a été étudiée chez des rats se comportant librement et traitées avec des antagonistes des récepteurs NMDA. Les déficits causés par les antagonistes ont eu une corrélation avec les déficits observés dans des études comportementales. Les résultats obtenus soutiennent l'idée que les récepteurs NMDA dans le système hippocampique servent d'intermédiaire entre l'internalisation des contenus et l'organisation de la nouvelle information environnementale et démontrent qu' ils ne sont pas pertinents pour la mémoire de travail spatial ou l'exécution de tâches une fois la représentation de l'environnement est stable. Les résultats suggèrent également que les représentations spatiales ressemblent plus à des relations multiples entre événements et non pas à des cartes topographiques de l'environnement. Pendant l'apprentissage, les représentations spatiales sont activées par des sous-séries plus petites de signales environnementales, lesquelles deviennent, éventuellement, suffisantes pour une navigation efficace. Ainsi, les représentations sont encodées comme des relations entre stimuli partageant des similitudes ou étant uniques à des événements particuliers. L'information nouvelle est organisée au moyen d'une plasticité synaptique dépendante de l'activation des récepteurs NMDA. Ce

mécanisme de plasticité pourrait ressembler aux changements neuraux observés dans la forme de potentiation au moyen d'un pulse intial suivi d'une rafale de stimulation de haute fréquence étudiée ici.

RESUMEN

El aprendizaje declarativo conlleva la internalización de información semántica y de eventos. Este tipo de aprendizaje depende de la integridad del sistema hipocampal. En roedores, el aprendizaje espacial es estudiado como modelo del aprendizaje declarativo. Para esta tesis, experimentos electrofisiológicos y conductuales evaluaron el rol de los receptores NMDA en la plasticidad sináptica y el aprendizaje y la memoria espacial de ratas. Una forma de fortalecimiento sináptico que conlleva potenciación mediante un pulso inicial seguido por una ráfaga de estimulación de alta frecuencia se estudió en ratas tratadas con antagonistas de receptores NMDA a las que se le permitió moverse sin restricciones. Los déficits causados por los antagonistas correlacionaron con los déficits observados en los estudios conductuales. Los resultados apoyan la idea de que los receptores NMDA en el sistema hipocampal median la internalización de los contenidos y la organización de nueva información ambiental, y muestran que éstos no son relevantes para la memoria de trabajo espacial o la ejecución de tareas una vez que la representación del ambiente es estable. Los resultados también sugieren que representaciones espaciales espaciales se asemejan más a relaciones múltiples entre eventos y no a mapas topográficos del ambiente. Durante el aprendizaje, las representaciones son activadas por subconjuntos cada vez más pequeños de señales ambientales, los cuáles eventualmente se tornan suficientes para una navegación efectiva. Las representaciones por lo tanto son codificadas como relaciones entre estímulos que comparten similitudes o que corresponden a eventos particulares. La organización de información nueva se da a través de una plasticidad sináptica mediada por los receptores NMDA. Es posible que este mecanismo de plasticidad se asemeje a los cambios neurales

observados en la forma de potenciación mediante pulso inicial seguido por una ráfaga de estimulación de alta frecuencia aquí estudiada.

ACKNOWLEDGEMENTS

I would like to thank Dr. Matthew Shapiro and Dr. Norman White for the opportunity to work with them, for their mentorship and their guidance.

For the studies that eventually led to this thesis and for the experiments presented here I received the assistance of several persons. I would like to thank Shari Mayerovitch, Irene Katsarkas, David Mutch, and Mathieu LeCorre for their excellent technical assistance. Thanks to Eric Hargreaves for discussions and his assistance in the electrophysiological experiments and to Denis Coté for helpful conversations.

I would like to thank Ms. Giovanna Locascio for her kind help in the thesis submission process. Also, thanks to A. Sebastián Mercado for the French translation of the abstract and his friendship. I would like to thanks my friends for their support and encouragement.

I am indebted to my parents and brother, whose constant support throughout the years has been crucial. Last, I would like to thank my wife Nuria Zayas for her friendship and patience. The completion of this project would have been very difficult without her constant support.

I dedicate this work to my children Sebastián and Isabel.

PREFACE

The study of spatial learning in rodents has shown that the hippocampal system mediates declarative learning and memory. Long-term potentiation (LTP) in the hippocampus is considered by some a physiological model of declarative memory formation. Although research relating LTP and memory formation has been very productive, there is no conclusive evidence that the mechanisms involved in the induction, maintenance, and expression of LTP are equivalent to the synaptic changes that subserve declarative learning and memory. Pharmacological interventions that affect both artificially induced synaptic strengthening and learning can be an effective strategy for relating them. For this thesis, I conducted a series of experiments to investigate the role of NMDA receptors in male Long Evans rats' spatial learning and synaptic plasticity. These studies were done as part of the research carried in Dr. Matthew Shapiro's lab at McGill University on the same topic. For all experiments pharmacological manipulations were done to evaluate rodent behavior or hippocampal synaptic plasticity.

In Chapter 2, I studied the induction of prime burst potentiation (PBP) in the perforant path-dentate gyrus pathway after the systemic injection of NMDA antagonists in freely moving rats. PBP, a short-term form of synaptic change similar to long term-potentiation except mostly in its duration, is obtained after high frequency stimulation of hippocampal areas where NMDA receptors are present. For the experiments, several doses of two drugs were used: MK-801 and NPC 17442, a non-competitive and a competitive NMDA receptor antagonist, respectively. MK-801 was chosen since it has been used to study spatial learning in our as well in other labs. NPC 17442, a lesser known drug, was used here more extensively to establish its role in learning and synaptic plasticity. This is the

first demonstration that PBP is affected in a dose-dependent manner by both antagonists, with higher doses causing faster rates of decay. The dose-response curves established in this Chapter served as a basis for the pharmacological manipulations done later in behavioral studies reported in Chapters 3, 4, and 5.

To study the relationship between the effects of these drugs on both PBP and spatial learning, I conducted a series of behavioral experiments. In Chapter 3, I studied the effects of the systemic injection of either NPC 17442 or atropine sulfate (a muscarinic cholinergic antagonist that has previously been shown to affect spatial learning), and the effects of the combined injection of low doses of both drugs, on spatial and cued learning in a water maze. Originally these experiments served as a continuation of a) studies done in my Masters thesis, where an animal model of the spatial learning deficits observed in Alzheimer's disease was evaluated by testing mice transgenic for a fragment of the human β-amyloid gene in the water maze, and b) a subsequent paper where hippocampal LTP and neuropathology were evaluated in the same transgenic mice. In the Masters thesis two hypotheses about the etiology of the declarative learning deficits associated with hippocampal damage observed in Alzheimer's disease were considered: deterioration of the cholinergic system and deterioration of glutamate neurons. Although the early literature reviewed supported both hypothesis, more recent studies point in the conciliation between the two hypothesis. Here I wanted to test the role of NMDA receptors in spatial learning and its possible interactions with cholinergic receptors of the muscarinic type. For this purpose, I carried two experiments to establish a dose-response curve for each drug that allowed to find the lowest dose that impairs spatial learning. These doses were then used in a third experiment with the objective of comparing subjects that received both drugs during the

same training session with subjects treated with only one of the drugs or the vehicle. This is the first demonstration that NPC 17742 impairs spatial learning in rats that previously were able to solve a cued task.

In Chapter 4, I did two studies using an 8/8 strategy on an eight arm radial maze to test rats given systemic injections of NPC 17742 during or after training. Previous studies assessing spatial learning after injections of MK-801 were used as a basis for the experimental paradigm. The idea behind this study was twofold. First, I wanted to measure learning driven by a qualitatively different motivational value than that used in Chapter 3. Although both the water and radial maze tasks measure spatial learning, the former has an aversive component while the latter relies on appetitive learning. This may affect the interactions of the hippocampal system with other brain systems for each task. Converging evidence from both tasks (i.e., results showing spatial learning impairments in both tasks after the same pharmacological manipulation) would support inferences regarding the nature of spatial learning in general and the role of NMDA receptors in this function. At the same time, the convergence of the results of these behavioral studies with that of the electrophysiological studies on hippocampal plasticity presented on Chapter 3 would in turn provide a more general knowledge of the cellular processes involved in hippocampal dependent spatial learning.

A second purpose of the studies in Chapter 4 was to evaluate the role of NMDA receptors in spatial working memory, which is measured in the radial arm maze. Here I evaluated whether spatial learning and working memory could be dissociated at the level of NMDA receptors by blocking them with a competitive antagonist. This is the first demonstration that a dose of NPC 17742 that impairs PBP and spatial learning in the water

maze also impairs spatial learning in the radial maze in a novel environment but not spatial working memory in a familiar environment.

The experiments in Chapter 5 were done with the goal of exploring which aspects of the environment are relevant for spatial learning. While most neurobiological studies using tasks for the evaluation of rats' spatial learning tend to focus on physiological manipulations (i.e., anatomical, pharmacological, proteinic, electrophysiological, or genetic), fewer studies rely on the manipulation of environmental cues. Some of these studies include the rotation of surrounding cues or the maze in relation to each other, the subtraction of cues, or variations in the place where rats are introduced to the apparatus. For this study, rats were extensively trained on the 8/8 radial maze task until reaching near errorless performance in an environment that consisted of a set of cues placed on surrounding curtains. After this, I introduced a structured set of manipulations of the environment, such as room content and geometry, and the organization of distal stimuli. A second set of manipulations included a mid-trial delay and changes in the location used to introduce rats to the testing environment.

Neither the interchange or removal of cues, changes in the geometry of the testing environment, a combination of both types of manipulations, nor delays affected performance. However, the introduction of new cues, the testing of rats in a novel environment, and the change in location used to to introduce rats to the room affected performance. Therefore, only manipulations that introduced new information either in the form of discrete distal cues or of a completely new room affected performance. Changes in already known information did not affect performance. This is the first demonstration that extensive manipulations of the environment impair solving of an 8/8 task in the radial maze only when new information

but not radical manipulations to already know information are introduced to the testing envirnoment.

Finally, to test the role of NMDA receptors in learning and performance of rats that had been extensively pre-trained, the minimal dose of MK-801 that affected PBP in Chapter 2 was administered and rats were tested in familiar and unfamiliar environments. The treatment affected new learning in an unfamiliar room, but not in the familiar room. The extensive spatial pre-training that these rats had received was not sufficient to override the effects of the drug during the learning of a new environment. The comparison of the performance levels of well-trained rats receiving MK-801 in a familiar and an unfamiliar environment shows that the impairments observed are due to the effects of the drug on the computations necessary to learn the spatial attributes of the environment and not on the procedures necessary to solve the task. Studies in Chapters 4 and 5 show that NMDA antagonists affect learning of novel environments regardless of the level of proficiency that rats have in the procedural aspects of the task (i.e., naïve rats in Chapter 4, well-trained rats in Chapter 5).

CHAPTER 1. GENERAL INTRODUCTION: The hippocampus and spatial learning and memory

The processing of space is mediated by a complex network of neural structures including the parietal cortex, the dorsal striatum, the core of the nucleus accumbens, and the hippocampal formation. This processing involves the perception of space; the encoding, storage and retrieval of spatial representations; spatial navigation; and the integration of spatial information with motivational and/or affective information. This thesis and the following discussion are concerned with the role of the hippocampal formation in spatial learning; therefore other structures and functions will not be discussed.

Various models exist regarding the exact role of the hippocampus in learning. The most prominent and therefore the most debated model posits that the hippocampus is essential for the learning of spatial representations and of the objects that shape it (Nadel, 1991; O'Keefe & Nadel, 1978; Zola-Morgan, Squire, & Amaral, 1986). Questions about the exact role of the hippocampus in spatial processing include whether it is involved in both spatial learning and spatial memory, whether spatial processing by the hippocampus is one of a wide variety of functions that depend on a more general processing or computational mechanism, and whether the hippocampus deals with spatial processing at all. The debate raises a more general question in both operational and theoretical terms: what is meant by spatial processing and spatial learning? Therefore, the relationship of the hippocampus and cognitive function, far from solved, is a matter of constant research and debate.

Spatial learning, due to both its complexity and flexibility and because it is a function shared by many mammals, is taken as an example of the processes that occur in cognitive learning. The processing of space requires the computation of the relationships among

several objects based on proximity and location. Moreover, this processing has to take into account possible changing environmental conditions such as the removal and shift of location of objects that define a place and variations of their characteristics, all without affecting the general map of a place or the navigational strategies associated with it. While behavioral paradigms have focused extensively on the learning of more or less stable environments, more research needs to be done on the processes that allow the construction of stable and useful representations of spaces that constantly are changing.

A. The study of the physiological basis of learning and the concept of memory systems

Research on the physiological basis of learning and memory has shown that these are not unitary functions. The contemporary assumption that there are memory systems is based partly on the study of anatomical regions that subserve different aspects of learning and memory. Researchers studying learning and memory brain systems have followed conceptualizations more or less along the lines of the psychological models originally proposed by James (1890), Bergson (1911/1949), and/or Ryle (1949), who in general terms proposed a distinction between conscious or symbolic-based recollections and automatic procedures or habits.

One trait of the contemporary conceptualization of memory systems is that they are relatively autonomous. Therefore, damage to a brain structure should not affect the functioning of another system, at least in substantial ways. This concept of autonomy follows loosely Fodor's (1983) concept of modularity. Fodor defined the manifestation of cognition as the integrated working of modules that subserve a central executive or general processing system. In his model though, modules are not defined in anatomical terms, but as

very specialized task-specific processes. According to this view each cognitive system would be characterized by qualitatively different types of computations.

As will be seen later, memory systems meet at least two criteria: they are autonomous from each other and are anatomically localized. Researchers came to these conclusions based on evidence from studies with both brain lesioned animals and neuropsychological patients.

1. The early study of an anatomical substrate for memory systems

Perhaps one of the first researchers to provide evidence that allowed the understanding of memory as a multidimensional function was Théodule Ribot (Finger, 1994). In 1881 he presented a survey of cases in the neurological literature on which brain damaged patients who did not have recollection of facts in a spatial and temporal frame were known nonetheless to remember skills and habits. Although he did not attempt to make a theoretical distinction between types of memory, Ribot speculated that "conscious" memories were associated with cortex, while habits and skills were related to the brain stem. He also advanced the idea that different forms of memory exist relatively independently from one another. The now widespread concept of memory systems is very similar to Ribot's findings (see Schacter & Tulving, 1994). However, the development of the concept of learning and memory systems was not popular until the mid-twentieth century. Meanwhile, most of the research on this area was carried from the perspective of conditioning phenomena (see Catania, 1992).

2. The search for the engram and the concept of organization

The modern idea that memory is not a unitary function was developed in the context of the study of conditioning. Although extremely fruitful and popular, conditioning models were not able to explain some learning phenomena. For example, Skinner's influential descriptive model (Skinner, 1934) did not fare well in explaining learning in the absence of a reward (Tolman, 1949), learning without stimulus hierarchy (Tolman, 1932, 1949), some evolutionary constraints in learning (García, Hankins, & Rusiniak, 1974; Lorenz, 1965), and language learning (Chomsky, 1959). Karl S. Lashley (1950, 1951) embarked on a project for the study of complex behaviors that arose partly as a criticism to conditioning models from a neuroscientific perspective. His main proposal was that behavior was organized in schemes of action represented at the neural level and that sensory information was processed by an active organism. Although these two concepts had already been explicitly addressed by European psychologists studying human development (see Piaget, 1964, 1967, and Vygotsky, 1991, for two examples of this), Lashley proposed them specifically in the context of the neuroscientific study of behavior and applied them to the study of memory.

Lashley argued for a strong empirical research program that would account for complex behaviors (Gardner, 1987; but see Weidman, 1999, for a contrasting view on this issue). In his interest of linking neural function to complex behaviors such as language, he rejected the notion of chains of conditioned reflexes or simple stimulus-response associations as the unique biological explanation for serially ordered behavior (Lashley, 1950, 1951). Instead, he argued that behavior should be organized at a level higher than that of simple associative chains. Syntax and slips of the tongue or the execution of an arpeggio are clear examples of this, since multiple actions are anticipated before a response is made or the

consequences of the action are experienced. Lashley (1951) held that any "input is never (processed) into a quiescent or static system, but always into a system which is already actively excited and organized" (p. 235). He proposed as an explanation for complex behaviors "the existence of generalized schemata of action which determine the sequence of specific acts" (p. 243).

Central to Lashley's research was his interest in the physiological basis of memory. Schemata would be represented in what he called an engram, which he would define as the memory of a hierarchical sequence of complex behaviors (Lashley, 1950). The term engram was originally coined by Richard Semon during the first decade of the twentieth century to refer to the trace that leaves the connection between stimuli (Finger, 1994). Lashley used the term loosely to refer to the representation of plans that guide complex behaviors. Instead of understanding organisms activity only as a sequence of responses to discrete stimuli, organisms spontaneously initiate many behaviors using internal representations somehow stored in the nervous system. Therefore, experience becomes represented in the nervous system not just as a catalogue of connections between an environmental cue and its consequent reaction, but as a plan to guide complex responses that might occur in parallel even in the absence of a stimulus that triggers that behavior (see Lashley, 1950, p. 170).

Although Lashley (1950) believed in the study of the neural basis of memory, he reached the conclusion that memories were not stored in strictly localized brain regions. In multiple experiments on which he removed areas of the brain of rats previously trained in mazes, he found no evidence of memory loss. Lashley concluded that memory is a function that is diffusely represented over the brain and was left with the impression that the engram was still elusive (see Lashley, 1950, p. 227).

Hebb (1949) developed Lashley's original notion of the engram. He hypothesized that memories are not located in individual cells. Instead, he proposed that a representation of an experience could be found in organized groups of cells that formed a circuit that might comprise different areas of the brain. Therefore, the localizationist idea originally proposed by Broca, Wernicke and others (see Young, 1970, and Finger, 2000) was revived in a much more complex way, one on which the brain represents information through the functional organization of its components. In this sense the idea of strict localization gave way to a flexible localization represented in a distributed network. This idea would imply that not one element or cell, but many, would be necessary for a particular memory to be formed. It allowed also for the idea that slight changes in the circuit could account for minute changes in memories, like slow degradation. Although these hypotheses had to wait several decades to be tested, they represented a new perspective of the neuroscience of memory. In particular, they had strong implications for the study of synaptic plasticity. In 1973 Bliss & Lømo reported that a particular pattern of stimulation was able to induce long-term potentiation of synapses in the dentate gyrus. This opened a new avenue for the research of the neural mechanisms that could be involved in the learning and storage of new information. This will be explained later.

Although very influential, Hebb's (1949) concepts were hypothetical and the physical substrate of memory remained elusive. Animal research during the first half of the twentieth century had not provided strong unequivocal evidence for a neural substrate for memory. However, research with humans showed some promising avenues for the study of the physiological basis of memory.

3. Early studies of memory systems in humans

A report by Scoville and Milner (1957) of a patient with severe anterograde amnesia, named H.M., usually is regarded as the most prominent early account of the role of a brain structure in learning and memory functions. Since this patient underwent bilateral medial temporal lobectomy, this prompted a series of studies involving this structure.

Patient H.M. had undergone this type of surgery to relieve epileptic symptoms. Although his epileptic condition improved, he was left with severe anterograde amnesia and limited retrograde amnesia (Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). In the first accounts of H.M.'s behavior, it was startling that even when he was able to remember remote events, he did not seem to have any recollection of events that had occurred in the past few minutes. Soon after the surgery it was evident that this patient had problems in remembering new information, but had few problems remembering events from his childhood and adolescence. Therefore he seemed to have a severe learning impairment. Since previous experimental searches for such a structure in rats had proven inconclusive, this proved to be a scientific milestone. H.M.'s case opened a door for the study of the physiology of learning and memory, focusing attention on the medial temporal lobes.

Before H.M.'s case, other similar accounts can be found in the literature. For example, in 1900 Bekhterev described a patient with bilateral medial temporal lobe damage who had impairments for storing recent events (see Luria, 1974). Despite this, historically the study of the temporal lobes as a structure critical for learning and memory in humans started to develop during the 1960's. During that time there were several reports of patients other than H.M. with damage to the temporal lobes, medial temporal lobes, or hippocampus who had memory impairments (DeJong, Itabashi, & Olson, 1968, 1969; Victor, Angevine,

Mancall, & Fisher, 1961). The wide variety of lesions underscored one of the intrinsic problems of the lesion analysis in neuropsychology: accidents or "nature experiments" allow for very informative (and terrible) but seldom specific impairments. Therefore, much of the study of amnesic patients was originally carried as single case studies.

The learning impairment found in patient H.M. and later in other patients led to at least two apparent theoretical developments in the psychology of learning and memory. One was regarding the relationship between short-term and long-term memory. Since H.M. had no major problems remembering remote information, and was able to keep in short-term memory new information for only a few minutes, it seemed at the time that the medial temporal lobe was necessary for encoding new knowledge into long-term memory. This was later replicated in patients with damage restricted to the hippocampus. For example, patients with hippocampal damage are able to repeat a sequence of digits after a very short delay (Cave & Squire, 1992), or have no difficulties in solving problems that require retaining verbal or visual non-verbal information for a short period of time (Cowey & Green, 1996). Since these patients do not have major problems with long-term memory either, the hippocampus does not seem to be necessary for storage. The hippocampus is rather necessary to learn this information for further use. At the time, this finding provided a neurological correlate to increasingly popular information processing models about the storage of information like that of Atkinson and Shiffrin (1971).

A second theoretical development resulting from the study of patient H.M. was related to the idea that there are qualitatively distinct types of memory, which led to the idea of neurologically dissociable memory systems. Although H.M. is not able to consciously remember events that happened after his operation, he can learn some information without

having a conscious recollection of the task performed, the objects used, the place where the experience occurred, when it occurred, or the people who administered the tasks. For example, he can learn to find a goal in a maze without remembering previously doing so (Corkin, 1965; Milner, 1965). He and other patients with similar damage are able to learn problem solving tasks such as the Wisconsin Card Sorting Test, perceptual classification tasks, motor coordination tasks, and priming of line drawings and patterns at a rate equivalent to that of normal control subjects (Corkin, 1968; Gabrieli, Milberg, Keane, & Corkin, 1990; Hamann & Squire, 1997; Milner, Corkin, & Teuber, 1968; Musen & Squire, 1992). Therefore, the learning impairment exhibited by these patient is specific to some type of information, specifically that which requires conscious awareness but not that which involves either cognitive or motor skills.

After the study of H.M. by Milner and colleagues (Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957) further studies with both humans and other mammals led to the conclusion that of all medial temporal lobe structures, the hippocampus seems to be responsible for learning functions. Furthermore, from all this research it was evident that the hippocampus is related to a particular type of learning, that is, declarative learning.

4. Two types of memory: Declarative and procedural knowledge

The study of amnesias of different etiologies, together with the study of some animal models amnesia, led to the view that memory is not a unitary function. Particularly useful has been the analysis of functional dissociations. For a behavioral impairment to be considered a dissociation it should involve the disruption of a task while there is normal performance in another task (Shallice, 1988). Each task should be within the same functional domain (e.g., learning, language, visual perception, etc.). The tasks should also

involve qualitative differences in the type of information that is processed and not differences in the level of difficulty needed to solve each task. This approach has led to the conclusion that learning and memory each work in modular ways, since subjects that have disruptions in brain functioning either of a permanent (e.g., because of a lesion) or temporary (e.g., through a receptor blocker) nature, and who are able to learn one type of material, are not able to learn other types of qualitatively different material across a variety of conditions.

Cohen and Squire (1980) developed a model to explain the learning dissociations observed in patients with medial temporal lobe lesions. As mentioned above, patients with anterograde amnesia due to damage to the hippocampal region or related structures are able to learn several skills and information without being aware of the experience. From this the researchers concluded that there are at least two qualitatively distinct memory types in the nervous system, one based on rules and procedures, and one based on propositional or data-based knowledge that was readily accessible to conscious recollection. Following the nomenclature originally proposed by Ryle (1949) they equated the first type with the knowledge of the semantic attributes of an object ("knowing that") while the second was equivalent to the knowledge of the procedures needed to solve a task or perform an action ("knowing how"). These two types of memory came to be known as declarative and procedural memory, respectively (Squire, 1987). Later procedural memory was also called "nondeclarative" (e.g., Squire & Zola-Morgan, 1991; Squire, 1992) to encompass learning that does not require conscious awareness or the use of any proposition, but that does involve a procedure that is not necessarily motor, such as priming and perceptual learning.

Declarative knowledge is understood as memory for both generic facts and events that have a spatial and temporal reference. Generic facts like the meaning of words or the

generally accepted use of objects is known as semantic memory. It is equivalent to the set of rules or characteristics that are common to a vast array of objects and that do not allow for the differentiation of a particular object from others in the same category. On the other hand, any information that makes reference to the direct experiences of the organism, and which can be contextualized in a particular place and/or time is referred as episodic knowledge. The semantic-episodic distinction was first proposed by Tulving (1972), who later modified this model referring to semantic memory as "knowing the present" in terms of the meaning of things and to episodic memory as "remembering the past" in terms of particular concrete experiences (Tulving & Markowitsch, 1998).

The declarative/nondeclarative distinction has proved to be a useful one. It offered a theoretical coherent explanation for an ever increasing number of observations that defied the traditional view taken by learning theories of the first half of the twentieth century that proposed conditioning (in its type S and type R learning variants) as the unique mechanism for the acquisition of new behaviors. Tolman (1949) and others had already observed that the behaviorists' scheme was restrictive and could not account for some serious anomalies (in the kuhnian sense) that the conditioning paradigms could not explain, suggesting that other types of learning should exist. In this case declarative knowledge can be differentiated from nondeclarative knowledge in the sense that the first is a cognitive system that allows for computations based on representations of the environment. These representations would consist of an organized relationship among stimuli or a cognitive map of the environment (Tolman, 1948). The map would be constructed through experience and would allow for flexible behaviors based on changing contingencies. This is similar to the proposals by Hebb (1949), Lashley (1950), Piaget (1967/1987; 1980), and Maturana and Varela (1972, 1979),

among others, in terms of considering a level of organization of behavior that is biologically ingrained and, as was explicitly stated by most of them, the product of experiences. Nondeclarative knowledge would be equated with nonconscious behavior, where no flexible plans or internal reflection are needed (see Tulving & Markowitsch, 1998). It is important to note that sometimes the terms explicit and implicit are used as synonyms for declarative and procedural, respectively (Schacter, 1987). Although there are subtle differences on the exact usage of the terms, the discussion of those is beyond the scope of this thesis.

B. Anatomy of the hippocampal system

The medial temporal lobes are necessary for the processing of declarative learning and memory (Scoville & Milner, 1957; Squire, 1987; Squire & Zola, 1996). The hippocampus, together with its connections, is one of the key components in this processing (Squire, 1992; Eichenbaum, Otto, & Cohen, 1994; Eichenbaum, Schoenbaum, Young, & Bunsey, 1996; Jarrard, 1993). Depending on the structures connected with the hippocampus, the nomenclature changes to refer to the hippocampus proper, hippocampal formation, or hippocampal system, which might create some confusion. Therefore it is relevant to establish a difference between these structures, specially since referring to one or another implies a distinct functional circuit (see Jarrard, 1991). The *hippocampal formation* comprises the *hippocampus* proper and other limbic structures. The hippocampal formation, adjacent cortical areas, and the connections between them comprise the *hippocampal system* (Cohen & Eichenbaum, 1993).

It is hypothesized that the distinctive nature of hippocampal information processing is due to the complex pattern of connectivity of the hippocampal system (Cohen & Eichenbaum, 1993; Eichenbaum, Schoenbaum, Young, & Bunsey, 1996). The system has

bidirectional connections with cortical as well as subcortical areas, sending information to cortical areas from which it received information in the first place, apparently for further storage. The structures within the system also sustain a complex pattern of connectivity among themselves, with some of these connections being reciprocal.

1. Anatomy of the hippocampus

The hippocampus proper is a C-shaped structure that projects from the septal nuclei to the posterior temporal lobe. This septotemporal projection is referred as the long axis. Perpendicular to this is the transverse axis.

The anatomy of the hippocampal formation has been interpreted in various ways according to the techniques used for its study. Simple observation of the structure led it to be called hippocampus, as it loosely resembles a seahorse. It was also called Ammon's horn, after the horns of the god of the sun Ammon depicted in some Egyptian frescoes. Systematic observations by Ramón y Cajal and by Lorente de Nó described in detail the structure of the hippocampus, noting that it has extremely well defined layers of multipolar cells. Ramón y Cajal divided this layer into two regions, regio superior and regio inferior. Lorente de Nó went further and subdivided the regio inferior into CA2 and CA3 (CA for Cornu Ammonis) and designated regio superior as CA1 (Johnston & Amaral, 1998). He added a CA4 region that referred to the hilar layer of the dentate gyrus (see below), which is directly connected to the hippocampus proper. This layer derived nomenclature has persisted nowadays, although the term CA4 is sparsely used. Collectively, these layers are known as the pyramidal cell layer.

More recent neuroanatomical techniques have led to a proposed functional organization of the hippocampus. Andersen, Bliss and Skrede (1971) suggested that the

structure could be divided into functionally autonomous transverse sections, called lamellae, each of which would include a portion of the dentate gyrus and all layers. According to this proposed organization, any stimulation of a small portion of the entorhinal cortex would stimulate a particular lamella (Amaral, Dolorfo, & Alvarez-Royo, 1991; Amaral & Witter, 1989). This hypothesis, known as the lamellar hypothesis, was derived mostly from electrophysiological studies that originally presented the hippocampus as a trisynaptic circuit. However, more recent neuroanatomical studies have modified this clean modular picture, describing complex interconnectivity among hippocampal regions. Using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin, Amaral and Witter (1989) studied the hippocampus and proposed an explanation based on the three-dimensional organization of the structure. They found that perhaps the only area of the hippocampus that is organized according to a lamellar explanation would be the dentate gyrus mossy fiber projection to CA3. All other areas project not only following the transverse axis, but also the septotemporal axis. This would mean that the activation of the hippocampus would not occur strictly in small lamellae but simultaneously in various areas along the septotemporal axis. In general terms however the trisynaptic view of the hippocampus continues to serve as a basis for current research (see Benes, 1999, for an example of this as applied to the study of neuropsychiatric disorders).

2. Anatomy of the hippocampal formation and the hippocampal system

The hippocampal formation consists of four main areas: the dentate gyrus, the hippocampus proper, the subicular complex, and the entorhinal cortex, which is regarded as associative cortex (Amaral & Witter, 1989). Of all these structures, perhaps the most studied one is the intrahippocampal circuit that serially connects its four main areas (Jarrard, 1991).

The hippocampal formation receives projections from various cortical regions via projections from the entorhinal cortex. The entorhinal cortex in turn receives information from other cortical areas through two other medial temporal areas: the parahippocampal gyrus and the perirhinal cortex (Insausti, Amaral, & Cowan, 1987). In general, the cortical areas connected to the hippocampal system receive information from higher order or associative cortices. This implies that the input received by the hippocampal system consists of information integrated from various sensory systems as well as information already processed by other systems.

The entorhinal cortex is a laminated structure that consists of six layers denominated from I to VI (for humans see Insausti, Tuñón, Sobreviela, Insausti, & Gonzalo, 1995; for non-human primates see Amaral, Insausti, & Cowan, 1987; for rats see Insausti, Herrero, & Witter, 1998). Mostly from layers II and II, the entorhinal cortex sends fibers to the dentate gyrus through the perforant pathway (Witter & Amaral, 1991). This pathway has two main regions, lateral and medial, which seem to have different physiological characteristics, the medial perforant pathway being more excitatory than the lateral (McNaughton, 1980).

The perforant pathway ends in the molecular layer of the dentate gyrus. The dentate gyrus is a U shaped structure which is composed of three layers: molecular, granule cell, and the hilus or polymorphic (a layer with cells of various types) (Johnston & Amaral, 1998). Hilar cells form association projections only inside the same layer. From the dentate gyrus information is sent to the hippocampus proper. There it passes through layers CA3 and CA1, which are interconnected mostly unidirectionally (see Amaral & Witter, 1989).

Information from the dentate gyrus goes directly to CA3 through mossy fibers. Layer CA3 cells form a set of fibers, the Schaeffer collaterals, that project inside CA3 and to CA1.

Layer CA1 has bidirectional connections with the subicular complex (Berger, Swanson, Milner, Lynch, & Thompson, 1980). This structure is comprised of three serially positioned regions: presubiculum, subiculum, and parasubiculum. Although they are connected in series, each of these regions has unique bidirectional connections with cortical and subcortical regions, which some see as a suggestion for a functional specialization of each subregion (e.g., Van Groen & Wyss, 1990). A circuit projects directly from the entorhinal cortex to the subiculum and layer CA1 (Jarrard, 1991). These project back to the entorhinal cortex, completing a circuit (Room & Groenewegen, 1985).

The hippocampal formation has output connections to other structures via three major pathways: precommisural fornix, postcommisural fornix, and nonfornical fibers, which collectively are referred as the fornix (Mark, Daniels, Naidich, & Hendrix, 1995; Swanson & Cowan, 1977). The hippocampus sends projections to the basal forebrain through the fornix (Aggleton, Friedman, & Mishkin, 1987). The structure allows the hippocampus to connect field CA1 and the subiculum to the lateral septum (Swanson, 1977). The fornix also allows the medial septal-diagonal band complex to project in turn to fields CA3 and CA4 of the hippocampus, the dentate gyrus, and the subicular complex. From the subiculum projections go to higher cortical areas, specially the prefrontal cortex (Charmichael & Price, 1995; Irle & Markowitsch, 1982; Leichnetz & Astruc, 1976; Swanson, 1981). The hippocampal system is connected to other subcortical structures such as the amygdala (Calderazzo, Cavalheiro, Macchi, Molinari, Bentivoglio, 1996; Herzog & Van Hoesen, 1976; Rosene & Van Hoesen, 1977; Saunders, Rosene, & Van Hoesen, 1988), the nucleus accumbens (DeFrance & Yoshihara, 1975; Groenewegen, Room, Witter, & Lohman, 1982; Krayniak, Meibach, & Siegel, 1981; Redish & Touretzky, 1997), the hypothalamus (Anschel, Alexander, &

Perachio, 1982; Raisman, 1970; Swanson, 1977; Swanson & Cowan, 1975); the supramammillary nucleus (Vertes, 1992), and the dorsal raphe nucleus (Andersen, Rigor, & Dafny, 1983).

C. Hippocampus, learning, memory and space

The study of the physiological basis of memory focused on the hippocampus after the original report about patient H.M. (Scoville & Milner, 1957). Some studies tried to recreate an animal model of the memory dysfunctions of this patient by lesioning the hippocampus and related structures. From the beginning it was apparent that the memory deficits obtained in these studies were not global, but specific to some tasks.

1. Research on humans

a) Role of the hippocampus in human learning and memory: Further evidence from patients with lesions restricted to the hippocampus

Although patient H.M.'s condition generated a large number studies regarding the role of the temporal lobes in human learning, the case does not provide clear cut evidence of the specificity of the hippocampal formation in declarative learning due to the extent of the damage. Recent imaging studies have corroborated the first account about the excised tissue reported by Scoville (Corkin, Amaral, González, Johnson, & Hyman, 1997). For example, the removal includes portions of the pyriform cortex which might be responsible for H.M.'s olfactory deficits (Eichenbaum, Morton, Potter, & Corkin, 1993). His surgery also includes a complete removal of the amygdala, which has been related to the processing of affective states (LeDoux, 2000). Few studies of the patient have addressed this last issue directly, although it has been reported that he has problems in spontaneously conveying affective information (Hebben, Corkin, Eichenbaum, & Shedlack, 1985). Also, probably associated

with the bilateral removal of the amygdala, the patient does not seem to mind unpleasant stimuli such as controlled electric shocks nor does he present normal galvanic skin response (Milner, Corkin, & Teuber, 1968). All these impairments and the extent of the surgery might cloud the issue of whether the hippocampus is solely related to the acquisition of new information.

The question of which medial temporal lobe structure is necessary for learning declarative information has received extensive treatment in the literature. The hippocampus seemed to be a candidate when some of the symptoms presented by H.M. were eventually observed in other patients with damage to the hippocampus (Muramoto, Kuru, Sugishita & Toyokura, 1979; Woods, Schoene, & Kneisley, 1982). More recent evidence suggests that damage restricted to the hippocampus or its main input or output fibers is sufficient to produce learning impairments similar to those observed in patient H.M.

Zola-Morgan, Squire and Amaral (1986) studied a patient, R.B., who due to ischemia had bilateral damage restricted to the hippocampus. Detailed post-mortem analysis showed that damage was limited to the pyramidal layer of field CA1. A lesion to this area causes a disruption in the flow of information from the hippocampus to cortical areas. R.B. had severe anterograde amnesia similar to though not as severe as that of H.M. Different from the later, R.B. had minor retrograde amnesia for some events that occurred one or two years before his lesion, and he had no other noticeable cognitive impairment. He performed poorly on several verbal and non-verbal learning standardized tests, and was also impaired in paired associate learning, story recall, and diagram recall when a delay was imposed but not when he was requested to recall the material immediately after exposure. Therefore, similar to H.M., R.B. had neither problems in short-term memory nor severe problems in memory for

remote events, but had an impairment in learning new information. Since other temporal lobe structures were spared in this patient it was concluded that the hippocampus contributes to the learning of new information related to facts and events. Studies of other patients with damage restricted to the hippocampus confirmed Zola-Morgan and colleagues idea (see Appendix A).

Studies that compare the performance of several patients with hippocampal damage due to ischemia have confirmed the findings with single case studies. For example, Hopkins and Kesner (in Kesner, Hopkins, & Chiba, 1992) assessed learning on 10 hypoxic subjects with damage to the hippocampus but with no apparent damage to the parahippocampal gyrus or temporal lobe. Patients performed poorly on a wide variety of verbal and non-verbal measures of the Denman Neuropsychology Memory Scale that are similar to those on which patients H.M. and R.B were impaired. Later Rempel-Clower, Zola, Squire and Amaral (1996) studied three additional patients with anterograde amnesia due to damage to the hippocampal formation. Detailed post-mortem analysis showed that all patients had loss of CA1 neurons, while damage to the rest of the hippocampal formation varied between each case. Two of the patients were as impaired as R.B., while a third had more severe anterograde amnesia. All of them, similar to patient R.B., had some form of temporally graded retrograde amnesia that went from very minor to severe.

The study of patients with damage restricted to the hippocampus has shown that within the medial temporal lobe, the hippocampus is necessary for declarative learning and lesions restricted to the structure are sufficient to produce deficits. This knowledge has been helpful in the understanding of neurodegenerative conditions that entail learning deficits, and has guided research on both neurological patients, such as Alzheimer's disease patients, and

normal humans with intact brains. For further discussion of these types of research refer to Appendix A.

b) The hippocampus and spatial learning in humans

The hippocampus plays a critical role in spatial learning in humans although other brain structures are also associated with spatial processing. Spatial deficits have been observed after damage not only to temporal but also to frontal and parietal cortices (Pandya & Yeterian, 1984). For example, patients with posterior parietal cortex lesions are impaired in solving tasks that require some knowledge about space (Postma, Sterken, de Vries, & de Haan, 2000). Frontal lobe patients are impaired in learning problem solving sequences based on spatial information (Vilkki & Holst, 1989). Normally, specialized regions within the frontal lobes process spatial working memory (Courtney, Ungerleider, Keil, & Haxby, 1996; McCarthy et al., 1994; Owen, Evans, & Petrides, 1996). It seems that the frontal lobes are implicated in the coordination of temporal sequences on a spatial task while the parietal lobes integrate visuo-spatial information (Villa, Gainotti, De Bonis, & Marra, 1990). In functional neuroimaging studies it has been observed that at different stages of an object-based selection task that uses spaces for orientation, occipital, temporal, parietal, and frontal cortices are active (Arrington, Carr, Mayer, & Rao, 2000). Together, this evidence shows that the circuit for the processing of spatial information is widely distributed over neocortex, and that spatial processing depends on the integration of highly specialized cortical modules that subserve functions such as the creation of a body map, attention, the location of objects, and the storage of a spatial map, among other functions. In general, information from sensory, visual and auditory primary cortices would converge in association areas, allowing for the formation of a map through the integration of different stimuli and the organization of

behaviors. Then, association cortices would project to limbic areas, via the entorhinal cortex. The limbic circuitry would be critical in the consolidation of spatial information, that then would presumably be stored in neocortex through limbic to cortical projections.

It seems that the neural circuit necessary for processing spatial information invariably involves the hippocampal system. The specific nature of the spatial memory impairment seen in patients with hippocampal damage seems to be in the acquisition of new spatial information and not in the use of already learned spatial information. So, patients with hippocampal damage can navigate and remember locations of places known before lesions occurred (Teng & Squire, 1999). For example, patient H.M. has spatial learning problems which indicates a role of the medial temporal lobe in spatial processing. This patient was not able to learn the location of a house where he lived after his operation; however, he had a good memory of the location of house where he lived prior to his operation (Milner, Corkin, & Teuber, 1968). In controlled studies, this patient was not able to learn tasks that require either visual or tactile spatial learning of a maze (Corkin, 1965; Milner, 1965).

The study of both neurodegenerative disorders and imaging data have contributed to the knowledge of the involvement of the human hippocampus in tasks related to every day functioning and the handling of information specific to humans. A discussion of this type of research can be found in Appendix A.

2. Research on non-human primates

a) Role of the hippocampus in non-human primate learning and memory

Many early non-human primate studies tried to create an experimental model of the amnesia observed in patient H.M. (Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). Although most non-human primate studies have been consistent showing that in

general the temporal lobe is implicated in learning and memory, they have produced mixed results regarding the specific temporal region critical for memory functions. Most behavioral tasks used to measure learning and memory impairments in non-human primates have focused on object recognition, specifically the delayed non-matching to sample task, where animals have to choose a novel object among a set of two. Sequences of pairs of objects are presented in the same location, each covering a food well, with only one being rewarded. After an animal chooses an object, another pair is presented which includes a novel, and now rewarded object, plus the object that was rewarded in the previous pair, this time without a reward. Choosing the rewarded novel object implies intact recognition.

Because researchers tried to create an animal model of the deficits observed in human patients with medial temporal lobe damage, initial reports of memory impairments in non-human primates included both the hippocampus and the amygdala as part of the memory circuit critical for recognition memory (Bachevalier, Parkinson, & Mishkin, 1985; Mishkin, 1978; Murray & Mishkin, 1984; Saunders, Murray, & Mishkin, 1984; Zola-Morgan & Squire, 1984, 1985). Some researchers tried to assess the contribution of each structure and size of the damage to the learning impairments observed. For example, Saunders, Murray and Mishkin (1984) observed in monkeys having either bilateral lesions in both structures, or bilateral lesions in one structure while unilateral lesions in the other, that deficits were related to extent of damage and not to the specific structure lesioned. Other studies in which monkeys had lesions to the hippocampus and/or amygdala produced contrasting evidence regarding the contribution of the hippocampus on object recognition. Zola-Morgan and Squire (1984) tested monkeys with both hippocampus and amygdala lesions, observing diverse patterns of impairments. While monkeys were impaired in discrimination tasks, they

learned tasks that required motor skills, similar to what has been observed in research with humans. Later they found that monkeys with lesions only to the hippocampus were impaired on delayed non-matching to sample (Zola-Morgan & Squire, 1986). This contrasts with other studies by Mishkin and colleagues (Mishkin, 1978; Parkinson, Murray, & Mishkin, 1988) where adult hippocampectomized monkeys performed as well as controls in object recognition tasks. A possible explanation for these differences might be that impairments in lesioned animals tend to disappear once they are trained before removal of the hippocampus (Mishkin and colleagues', but not Zola-Morgan and colleagues' technique; see Ringo 1988). Later, it was found that monkeys with amygdala lesions sparing the hippocampus and temporal cortical areas are able to learn object recognition tasks normally (Gaffan, 1994; Parkinson et al., 1988; Zola-Morgan, Squire, & Amaral, 1989b). In general, differences in developmental and experimental design as well as in the particular structure removed did not allow a clear understanding of the exact role of the hippocampal system in declarative learning.

Further research has shown that some cortical areas surrounding the hippocampus are relevant for declarative learning in non-human primates, essentially the parahippocampal, perirhinal, and entorhinal cortices (Zola-Morgan, Squire, Alvarez-Royo, & Clower, 1991; Zola-Morgan et al., 1989b; Zola-Morgan, Squire, Amaral, & Suzuki, 1989). The hippocampus is heavily connected to the perirhinal and parahippocampal cortices through the entorhinal cortex (Suzuki & Amaral, 1994). The perirhinal and parahippocampal cortices receive projections from various sensory processing cortices. Combined lesions of both structures produce impairments in delayed non-matching to sample, concurrent discrimination, and object discrimination, but not in pattern discrimination (Zola-Morgan,

Squire, Amaral, & Suzuki, 1989). The perirhinal cortex contributes to hippocampal processing since combined lesions of both structures cause larger impairments than lesions restricted to the hippocampus (Alvarez, Zola-Morgan, & Squire, 1995; Zola-Morgan, Squire, Clower, & Rempel, 1993; Zola-Morgan, Squire, Rempel, Clower, & Amaral, 1992). These impairments are polymodal which suggest that these cortical areas are crucial for the processing of global information (Suzuki, Zola-Morgan, Squire, & Amaral, 1993). Since the perirhinal cortex provides input to the hippocampus, lesions restricted to the structure suffice in producing deficits in delayed non-matching to sample (Meunier, Bachevalier, Mishkin, & Murray, 1993). In general terms, it seems that as more cortex adjacent to hippocampus is lesioned more severe memory impairments occur. For example, monkeys with hippocampal, entorhinal, parahippocampal and perirhinal lesions are more impaired than monkeys with lesions to all these cortical areas except the perirhinal cortex (Zola-Morgan, Squire, & Ramus, 1994).

Although most researchers would agree that damage to cortical areas contributes to deficits that arise from hippocampal damage, there is no agreement regarding whether this contribution is merely of a quantitative or more of a quantitative nature. In general though, Squire and Zola-Morgan (1996) identify the medial temporal lobe with a declarative memory system. Their research shows that while in humans damage restricted to the hippocampus proper is sufficient to cause declarative learning impairments, in monkeys damage that extends to surrounding cortical areas exacerbates declarative learning impairments (see Meunier, Hadfield, Bachevalier, & Murray, 1996, and Nakamura and Kubota, 1996, for differing explanatory models).

b) The hippocampus and spatial learning in non-human primates

The introduction of a spatial component to a learning task is correlated with the firing of hippocampal neurons (e.g., Wilson, Riches, & Brown, 1990). Electrophysiological research on monkeys has shown a role of the hippocampus in the processing of spatial information.

Studies of the spatial location of objects have been carried out in both freely moving monkeys and monkeys staring at the location of an image on a computer screen. Both methodological approaches have shown that hippocampal cell firing is correlated with the location of an object or image. Rolls and colleagues (1989) found both hippocampal cells that fire only to the location of any image on a specific part of a screen and cells that fire only when a specific object is located in a specific area of the screen. During a task where monkeys were shown an object and after a short delay were presented either with the same or a different object in the same or a different location, Cahusac, Miyashita and Rolls (1989) recorded hippocampal cells that consistently respond to specific objects shown on specific locations of a computer screen. They also found that some of these neurons fired during specific moments of the task: either upon initial presentation, during the delay, or during the second time they were shown the object. This suggests that hippocampal neurons are involved in different stages of visual information processing.

In non-human primates hippocampal neurons seem to process information related to the displacement of the animals through an environment. It has been observed that hippocampal neurons fire either while monkeys sitting on a cart move on a test chamber with visual cues or rotate on their own axis in the same place (O'Mara, Rolls, Berthoz, & Kesner, 1994). When visual cues are removed, some cells continue to fire upon movement.

However, other neurons fired when the chamber was rotated or moved from or towards the monkey while the subject remained in the same location and position. It seems that some hippocampal neurons are driven by vestibular input while others are activated by visual stimuli (O'Mara et al.). Both of these aspects seem to be relevant when assessing the role of the primate hippocampus in spatial processing.

Finally, the parahippocampal cortex seems to be involved in spatial functions similar to those of the hippocampus. Both, hippocampal and parahippocampal neurons fire when freely moving monkeys stare at a particular place in the environment independent of where the animal is located or the direction of its head (Rolls, Robertson, & Georges-François, 1997). Moreover, when visual cues are made opaque or lights turned off these cells keep firing when the animal looks at the same place in the environment (Robertson, Rolls, & Georges-François, 1998).

3. Research on rodents

Rodents, especially rats, have been extensively used to understand the physiological basis of learning and memory using a variety of techniques including behavioral analysis, lesions, neuropharmacology, electrophysiological recordings, and, in mice, genetic manipulations. Several authors have tried to synthesize this voluminous research in impressive ways (e.g., Cohen & Eichenbaum, 1993; O'Keefe & Nadel, 1978). The discussion here will not attempt to emulate such accomplishments, and only some of the most relevant and illustrative studies regarding the role of the hippocampus in learning and memory will be discussed.

a) Early research on hippocampal functioning in rats

In the early literature on hippocampal function lesions figure prominently as one of the preferred methods for the study of this structure. Although rodent studies did not necessarily seek to produce a model of the impairments observed in patients with medial temporal lobe (such as H.M.) as a primary goal, early results contrasted markedly with those in the human literature. Also, compared with research on non-human primates, which had produced mixed results, lesions on rats met a different set of problems. While lesions of the hippocampus in non-human primate research varied widely in terms of the exact anatomical location and on the inclusion of other temporal lobe structures, many of the difficulties of the research conducted with rodents derived more from the approaches followed to assess behavior and the assumptions made about the cognitive systems tested.

In the first rodent studies of the effects of hippocampal damage contemporary assumptions about learning and memory led to the design of behavioral tasks that tested S-R learning abilities. The literature on learning up to the 1950s had followed two major experimental approaches, that of verbal learning and that of conditioning tasks (Catania, 1992). These approaches did not take into consideration the possibility of qualitatively different types of learning. As is now known learning in conditioning paradigms however does not depend on the integrity of hippocampus.

Early experiments with rats showed, perhaps counter-intuitively, that lesions to the hippocampus improved rather than impaired the learning of some tasks. For example, in an active avoidance task using an apparatus with two adjacent compartments, hippocampal lesioned rats learned faster than controls to move from one compartment to another when a sound that anticipated a foot shock was produced (Issacson, Douglas, & Moore, 1961). In

operant conditioning tasks, where a simple response is needed to obtain a reward, hippocampal lesioned rats learned faster than controls to perform the task (Schmaltz & Isaacson, 1967). More recent studies have shown functional dissociations where lesions of the hippocampal system improve the learning of several tasks, while impairing others (e.g., McDonald & White, 1993).

b) Models of hippocampal functioning in rodents

i. The hippocampus as a cognitive map

One of the most suggestive theories about mammalian hippocampal function is that it serves as a cognitive map. This means that the hippocampus mediates the creation of a representation of the environment so that an organism can navigate efficiently using general aspects of a particular environment. This idea was put forward by O'Keefe and Nadel after reviewing a large body of research on hippocampal function (Nadel & O'Keefe, 1974; O'Keefe & Nadel, 1978). Much of the research they considered did not fit into a coherent theory. These authors concluded that the hippocampus is necessary for spatial learning. Other types of learning are dependent upon other brain structures. This theoretical model supported the concept that memory is organized as multiple systems. O'Keefe & Nadel used rats' navigational strategies to provide descriptions of the types of information mediated by the systems. Mammals use at least two kinds of strategies for navigating through an environment: taxon and locale. Taxon strategies are those in which an animal orients its movements using a single stimulus. Locale strategies describe displacement guided by an array of environmental stimuli in the form of a map.

The idea of learning through a cognitive map was originally put forward by Tolman (1948). According to Tolman a map is a representation that an organism forms of the

environment in which the perception of an array of stimuli consists in the organization that the organism imposes on stimuli in respect to one another. In this map no specific stimulus is necessarily more relevant than another, so the absence, modification, or change in position of one or several individual stimuli should not affect the general quality of the map. Navigational behavior thus depends on space as a whole and not on a particular stimulus. Acquisition of this information is not necessarily due to the search for a reward, but might be part of a general exploratory tendency. Mammals' behavior therefore does not have to depend always on environmental stimuli that have a particular incentive value, as was the case in other theories like that of Hull (1943, 1951). For O'Keefe and Nadel, the hippocampus is necessary for adaptive behaviors since it allows mammals to navigate efficiently according to a representation of the relationship among stimuli in the environment.

Nadel and O'Keefe (1974) analyzed several studies of hippocampal function finding them lacking a comprehensive explanatory account. For example, they found puzzling that the hippocampus serves to transfer the recollection of information from short-term to long-term memory, yet some information, like the learning of habits, goes into long-term memory even when the hippocampus is removed or damaged. However, they found that animals with damage to the hippocampus were not able to learn tasks that depended exclusively on spatial cues.

O'Keefe & Nadel's (1978) theory was congruent with a study by O'Keefe and Dostrovsky (1971) where they recorded neuronal activation in the hippocampus. Rats were implanted with recording electrodes in the hippocampus and placed in an enclosed controlled environment defined by several cues. The authors observed that some neurons fired only

when the animal was in a particular place or its head was oriented toward a particular area of the environment. This suggested the idea that some cells in the hippocampus are involved in the creation of a representation of the environment that could be considered a spatial map. The cells that behaved in such a particular way were called place cells.

In a review of his and O'Keefe's model, Nadel (1991) summarized the most important points of the spatial map theory of the hippocampus. First, the hippocampus is necessary for a spatial memory system and not for the general processing of spatial information. Second, the hippocampus is only one of the many brain systems that mediates different aspects of the processing of space. Finally, the original theory proposed as a hypothesis that the left hippocampus represents things other than actual physical space. Regarding the first point, the theory restricts the scope of hippocampal function to learning. For example, it has been observed that lesions to the hippocampus only affect working memory based on allocentric information, but does not impair allocentric distance discrimination (Long & Kesner, 1996). The second consideration admits the possibility that other brain structures either receive or send spatial information to the hippocampus (see Friedrich, 1990). This would mean that the processing of space is due to a complex brain circuit. The last point was originally presented as a hypothesis, mostly to explain than in humans the hippocampus might have become specialized to also process verbal information. Nadel (1991) states that enough evidence has accumulated to formulate such a theory (see Appendix A for details regarding this matter).

The spatial memory theory has been supported by other studies. For example, Morris (1981) devised a task to test the idea that rats indeed use a spatial map to navigate. In what is known as a water maze task, Morris placed rats on a tank filled with water to search for a

hidden platform submerged under opaque water. The tank is placed in a room and is surrounded by multiple cues. Since the platform is hidden in this task, rats have to find its location based on its relation to other stimuli in the room and not based on the visual attributes of the actual target stimulus. Several sessions are given in which a rat has to swim to the platform to escape the water. Rats learn this task quickly as measured by the reduction of the time it takes to reach the platform. Rats with hippocampal damage cannot perform this task (Morris, Garrud, Rawlins, & O'Keefe, 1982). Although there is a reduction in latency, eventually improvement in performance stops and remains significantly worse than that of control animals. This deficit could be attributed to a general perceptual or motor impairment if no other task were given to rule out that possibility. However, in a cued version of the task where the platform is not hidden, rats with hippocampal damage are able to swim directly to the platform, not significantly different from normal rats. The comparison between the spatial and cued versions of the task establishes a functional dissociation. The hippocampus therefore is important for learning of spatial representations but is not necessary for the learning of S-R associations, at least in the water maze paradigm.

ii. The hippocampus and working memory

The idea that the hippocampus is important for working memory derives from experiments with rodents. In general the concept of working memory refers to the ability to process information in real time. It is related to the concepts of primary memory originally proposed by James (1890) and of short-term memory proposed by Atkinson and Shiffrin (1971). Working memory though refers not merely to the temporary storage of information, but to a system where information is rapidly processed for use, encoding or forgetting.

When considering the concept in terms of the operations involved in its processing and expression, the literature on rodents differs markedly from that on primates (both human and non-human). In humans, Baddley (1996) defines working memory as a process that depends of two sub-systems (the phonological loop and the visuo-spatial sketch pad) that fragment and process information independently, sending it then to a central executive that relates it to both previously stored and incoming information for further use, all within a period of time that lasts from seconds to few minutes. In terms of primate anatomy, working memory has been related to the integrity of the frontal lobes, mostly the prefrontal cortex (Goldman-Rakic, 1996). In rodents though the concept derives from studies of lesions of the hippocampus. Olton and colleagues (Olton, Becker, & Handelmann, 1979) conceptualized working memory as the temporary storage of information during one trial, a period after which it would be forgotten. In this case information can last for the duration of the trial, which in some experimental paradigms can be several hours. The variants of the working memory concept are non-contradictory, nor are they due to species differences. They simply refer to operationalizations of the functions of two different anatomical structures. Hereafter, the concept will be discussed as it refers to the functions of the hippocampus.

Olton et al. (1979) contrasted working memory and reference memory, which is the information needed across trials to solve a task and that could be an operational equivalent of long-term memory. To assess both types of memory Olton and colleagues devised an apparatus known as the radial arm maze. In this task, arms (usually eight) extend from a central platform that is surrounded by doors that give access to each arm. A piece of food is placed at the end of each arm and is not replaced after it is eaten by a rat on the maze. Therefore, within each trial a rat must remember which arms have been entered using

environmental cues for reference (hence the term reference memory). Working memory accuracy is tested by measuring how many times a rat re-enters arms after it has already eaten the food there. The task can be varied so that all or some arms are baited. In the variation of the task where only some arms are baited, reference memory accuracy is tested by measuring how many times a rat enters an arm that has never been baited (Olton & Papas, 1979). In the variation of the task where all arms are baited, a rat has to remember *when* it should go to that arm, meaning that the animal has to retain intra-trial information of all the visited arms (Olton et al., 1979). In both variations, Olton and colleagues observed that rats with hippocampal damage made significantly more working memory errors than controls. This led them to conclude that, at least in the radial arm maze, the hippocampus is important for temporarily processing information during one trial, discarding it once it is used, and acquiring novel information in the next trial.

The Olton account added a new dimension to the understanding of hippocampal function. The hippocampus had been understood as a structure important for the storage of new information into long-term memory. Now another closely related function was assessed. Furthermore, they showed that these functions were dissociable depending on the time when a lesion to the hippocampus was made. Olton and Papas (1979) trained rats on a 17-arm radial maze where only eight arms were baited. After the rats acquired the task, some received lesions to the fornix and after recovery all were retested on the same apparatus and environment. Although initially rats with fornix damage were impaired, eventually their ability to visit only the baited arms improved. However, they made considerably more working memory errors than controls. In this case, information that already had been learned was not affected by lesions to the fornix, although they were not able to acquire new trial

specific information. The authors assumed that fornix and hippocampal lesions are equivalent, but this assumption has since been questioned (eg, Barnes, 1988; Chai & White, 2004).

Another finding from Olton and colleagues was that working memory does not necessarily depend on spatial cues. Olton and Fustle (1981) trained rats on an elevated four-arm radial maze that had salient visual and tactile cues only within each arm. To reduce the possibility of using spatial navigation as a strategy to solve the task they eliminated distal cues by covering the arms with a cloth and switching the positions of the arms between trials. As in the Olton and Papas (1979) study, rats were trained and then operated. Normal rats were able to learn the task. Rats with lesions to the fimbria-fornix however performed at close to chance levels.

In conclusion, similar to working memory as conceptualized by Baddeley (1996), working memory in Olton and colleagues' accounts refers to a flexible mechanism for the processing of both spatial and non-spatial information. This flexibility means that although some information remains constant during a learning session, other information changes constantly and the hippocampus is important for this processing. A good adaptive strategy therefore would require both intact working and reference memory mechanisms.

iii. The hippocampus and the establishment of configural associations

Another model of hippocampus function as it relates to learning about the environment posits that the structure is important for establishing specific relations among a diversity of stimuli (Sutherland & Rudy, 1989). This is in contrast to the learning of one-to-one associations between stimuli, such as stimulus-stimulus (i.e., Pavlovian) or stimulus-response associations. In this model, a rat would pair a response to an array of stimuli

organized in a particular manner, not just to the simple occurrence of the stimuli. In this sense, the hippocampus is necessary for establishing a configuration among stimuli. According to the model, the hippocampus would solve a task that other systems are unable to solve by associating simple stimuli. The model acknowledges that there is more than one memory system.

The model is congruent with other models that propose a role for the hippocampus in spatial learning. However, configurations are not limited to spatial information. Space is only one example of a configuration. A configuration would be defined in this case by a situation in which an animal has to eliminate ambiguity of a particular cue based on its relationship to other stimuli (Sutherland & Rudy, 1991). In a negative patterning discrimination a rat learns to respond to either a light or a tone, as well as it learns not to respond to both stimuli presented together. Rats with hippocampal damage cannot solve this task. Therefore, the authors conclude that the hippocampus eliminates ambiguity from a context, even when this context is not defined in spatial terms. Context is defined in associative terms: a context is a configuration of stimuli associated in a network (Rudy & Sutherland, 1994). Several stimuli are associated as a set and they would elicit a particular response that would not be elicited by only one of the stimuli of the set.

In this account, the hippocampus does not necessarily process information of a qualitative nature different from other systems, such as in the declarative-procedural distinction. The difference here between associations would rather be a quantitative one where associative learning would require the association between one stimulus and another, while configural learning would require the association of one stimulus with a set (i.e., more than one) of stimuli. Ambiguity would be solved according to a specific pattern where, even

when there is an overlap of stimuli, the difference established among patterns depends on the exact stimuli in the set. Although no stimulus is more relevant than any other, the model does not make any explicit claim about whether the absence of a cue or a particular subset of cues would disrupt an already learned behavior.

iv. Hippocampal processing in the context of memory systems: Some limits of the functions performed by the hippocampus

The models discussed above share the assumption that the hippocampus mediates a specific type of cognitive learning. Either implicit or explicit in them is the notion that there is more than one memory system in the brain. However, the main aim of these models is to provide an account of hippocampal functioning and not of other memory systems. In methodological terms, many of these models follow the approach of studying single dissociations to explain what the hippocampus does and what it does not do. Since they do not examine damage to other structures, these models do not pretend to explain what brain systems are involved in other types of learning not affected by hippocampal damage, nor do they pretend to explain any possible interaction between these structures in terms of the behavioral manifestations of this interaction. To do this would require the study either of dissociations that present a pattern of deficits opposite to those observed after hippocampal damage, or the study of multiple dissociations. In this last approach the behavior of organisms that sustain lesions to one brain system is compared to that of other organisms with lesions to different systems. As explained before, the behaviors affected by each lesion should fall along one functional domain.

Several researchers have compared the behavioral consequences of lesions to the hippocampus or fornix to those that occur after lesions restricted to the dorsal striatum or lateral amygdala. This research provides an anatomical account of several brain memory

systems as well as an understanding of the interactions that can occur among some of those systems. One of these studies is of a double dissociation between hippocampus and caudate nucleus (Packard & McGaugh, 1992; Packard, Hirsh, & White, 1989). While lesions to the fornix do not impair tasks that require simple S-R associations, lesions to the caudate nucleus do. In a variation of the standard eight arm radial maze task that tests S-R learning, referred as a win-stay task, rats are required to choose four rewarded randomly selected arms that are lit with light bulbs on the entrance to the arm (Packard, Hirsh, & White, 1989. Rats with lesions to the caudate nucleus cannot establish an association between the lit arm and an appropriate response, choosing rewarded and non-rewarded arms indistinctly. Interestingly, rats with fornix lesions are more accurate than controls. On the standard version of the task, or win-shift task, where all arms are baited and no arms are lit, animals are required to visit each arm only once. In this task, fornix lesioned rats were impaired, while normal and caudate lesioned rats were able to solve the task.

Similar to the results by Packard et al. (1989), Kesner and colleagues observed several dissociations that involve the hippocampus as well as other structures (Kesner, Bolland, & Dakis, 1993). They compared lesions to the hippocampus, caudate nucleus, and extrastriate cortex finding that each structure is necessary for only one type of learning. In this case, they found that the hippocampus is important for spatial learning while the caudate is necessary for the association of a stimulus with a response.

These studies explain the dissociations observed in animals with hippocampal damage. It might be argued that in general animals with hippocampal damage are able to solve cued but not spatial tasks because the former are easier than the latter. If this were true, then the argument for functional specialization might be weak. However, if learning

deficits other than those considered declarative are present after damage to a non-hippocampal structure, as in this case the caudate nucleus, then the argument that there are qualitatively different memory systems is compelling. Furthermore, the fact that hippocampal lesions improve performance in S-R tasks suggests that the memory systems are independent.

The hippocampus also seems to also interact with the amygdala. In terms of learning, the amygdala is necessary for forming stimulus-reward associations (Hiroi & White, 1991). The dissociation between functions depending on the amygdala and hippocampal system have been studied by several researchers (Kesner & Williams, 1995; McDonald & White, 1995; White & McDonald, 1993). One example has made use of retention tasks (Kesner & Williams). After learning to discriminate between two reinforcements with different concentrations of sugar, rats received lesions either to the amygdala or hippocampus. Rats with lesions to the amygdala were severely impaired in choosing reinforced objects while rats with hippocampal lesions presented no deficits at any of several delays. Another study with similar results is one where rats trained on a conditioned place preference task learn to prefer one arm over another of a radial arm maze (White & McDonald, 1993). In this variation in the use of the radial arm maze distal cues are used and the maze is rotated with the intention of making the task dependent on spatial stimuli. However, although the task has a spatial component, rats have to learn an association between stimuli and a reward. White and McDonald made lesions restricted to the fornix, lateral amygdala, or dorsal striatum, and combined fornix-amygdala lesions. After training, they observed a distinctive pattern of behavior for each group. Amygdala and fornix-amygdala lesioned rats were impaired in the task, dorsal striatum lesioned rats acquired the task as well as controls, and

fornix lesioned rats showed larger preferences. In this case, as in the Packard et al. (1989) study assessing caudate and hippocampal functions, the processing of spatial information interfered with a type of associative learning, in this case, a stimulus-response association: when the spatial learning was eliminated, performance on the stimulus-response task improved.

Together these studies show that even when some aspects of a task might involve spatial learning or working memory components hippocampal damage has no negative effect when the computations necessary to solve the task are not mediated by the hippocampus. The hippocampus therefore is not important for stimulus-reward associations, but for the processing of contextual information. This has been observed also in fear conditioning, where lesions of the dorsal hippocampus impair the ability of rats to learn a contextual, but not a cued conditioned fear task (Phillips & LeDoux, 1992).

In conclusion, even when memory systems are autonomous, mammals might solve some tasks using one of several possible strategies dependent on various memory systems. At other times a learning experience might involve the simultaneous processing of qualitatively different types of information, some of which might interfere with one another, as observed in studies where facilitation on some tasks occur after lesioning a particular system not necessary for solving the task. In the studies considered here, the hippocampus figures prominently as such a structure. Even when it is not necessary for solving some tasks, the hippocampus still processes information in parallel with other structures relevant for solving a task, modulating the final outcome of a particular behavior. The hippocampus seems to be a structure necessary for relating information of different sorts while other structures such as the amygdala or the striatum are in charge of less flexible behaviors.

c) Electrophysiological correlates of hippocampal functioning

Electrophysiological techniques are often used as an approach to understand hippocampal function. Most studies employing these techniques seek to understand synaptic plasticity after environmental, experiential or invasive manipulations. The ultimate goal of many of these studies is to present a model of how synaptic function changes over time to represent particular aspects of experience. Two of the most common techniques for this study are the recording of place cells and the induction of long-term potentiation.

i. Place cells

The study of place cells is directed at understanding how cellular components of the brain are involved in the processing of a spatial representation. A characteristic of hippocampal pyramidal cells is that they fire in a specific pattern in relation to the location of a rat in a well-known environment. The cells that show this pattern are called *place cells*, while the location where they fire is known as a *place field* (O'Keefe & Dostrovsky, 1971; Speakman & O'Keefe, 1990). The firing pattern is in the form of a complex spike, which is characterized by bursts of rapid firing when the animal is in a particular location (Ranck, 1973). The complex spike pattern of these place cells has been recorded in CA1, CA3, and the dentate gyrus (McNaughton, Barnes, & O'Keefe, 1983). Cells in the latter region have been identified as "theta cells", meaning that they fire at a rate of approximately 7/sec during exploratory behaviors (Rose, Diamond, & Lynch, 1983).

The firing of place cells does not seem to be related to the movements of an animal or to any goal that might be present (such as a food pellet). In rats trained to find two different goals in the same task in respect to the same set of cues, place fields do not change with respect to the goal and keep firing in relationship to the cues (Speakman & O'Keefe,

1990). The firing pattern of a single cell is specific to a particular location in a given environment. The same cell might respond to another location within the same or a different environment, but the firing pattern for that location is different (O'Keefe & Conway, 1978). Therefore, it is not necessarily only the cell but also it's firing pattern that determines the neural encoding of a location.

The removal of cues in a controlled environment after place fields have been established shows that some cells might respond to a few or even one cue instead of to the whole relationship among the cues (Muller & Kubie, 1987; O'Keefe & Conway, 1978). Removal of all cues has shown that cells continue to fire in the same location, possibly due to path integration of movements required to reach the location (Knierim, Skaggs, Kudrimoti, & McNaughton, 1996; Smith, 1997), or to unidentified static cues (O'Keefe & Speakman, 1987). Overall, the observation of the phenomenon of place cells suggests that the hippocampus mediates the coding of spatial experience in an organized manner that is stable over time.

ii. Long term potentiation

Place cells represent a long-term change in brain function after experience in which several aspects of space are encoded in the particular firing of cells. The construction of such a representation most likely entails a change in synaptic plasticity, where new patterns of neuronal activity encode experience. Since Bliss and colleagues (Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973) reported that a specific pattern of stimulation (commonly known as tetanus) of the rabbit's perforant path produced a long lasting change in neuronal response in the dentate gyrus, LTP has been considered by many as a possible mechanism for understanding some of the processes involved in hippocampal-related

memory formation. Originally, and from a mostly intuitive or logical point of view, Ramón y Cajal (1941) had proposed that for experience to be remembered at least some kind of synaptic change must occur. Later, Hebb (1949) proposed a similar but much more elaborate idea in what is currently known as Hebb's postulate or rule of synaptic efficacy. In a much quoted statement, Hebb proposed that:

> "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased." (Hebb, 1949, p. 62).

Hebb hypothesized that a single neuron could not account for the development of a perceptual representation but that these must entail the operation of an assembly of neurons, where new functional patterns arise through the strengthening of connections. The persistent activation of one neuron by another neuron would somehow produce a change in "synaptic knobs". These ideas remained largely hypothetical until studies by Bliss and colleagues in 1973 suggested the possibility of studying physiological mechanisms of synaptic change.

LTP has been induced both in vivo and in brain slices. In vivo it has been studied in both deeply anesthetized and freely moving animals and it has been observed to last from a few hours to several weeks. Regardless of the type of preparation used, LTP in the hippocampal system is usually induced almost immediately after a brief high stimulation exerted on an afferent fiber (Brown, Chapman, Kairiss, & Keenan, 1988) and does not seem to vary between preparations (Teyler, Alger, Bergman, & Livingston, 1977). It's production requires both high frequency stimulation and a stimulus strength that surpasses the normal threshold intensity (Nicoll, Kauer, & Malenka, 1988). The required strength is determined

by a summation of excitatory postsynaptic potentials (EPSPs). This is attained by both presynaptic stimulation and simultaneous postsynaptic depolarization (Brown et al., 1988).

LTP is usually measured as a strengthening in synaptic connectivity in populations of neurons. This strength can be measured in at least two ways. One is to measure the time it takes for a response to occur once a pathway is stimulated. Since potentiation facilitates neuronal response, as seen by a rapid increase in depolarization, the EPSP slope changes. Another measure is a recording of an increase in the intensity of the response, as indicated by an increase in the amplitude of the population spike after tetanic stimulation.

LTP has been recorded in several areas of the brain and in the hippocampal formation three projections have been widely studied: the projection coming from the entorhinal cortex to the dentate gyrus granule cells via the perforant path, in mossy fibers that project from the dentate gyrus to CA3, and in the Schaeffer collateral pathway that projects from CA3 to CA1 pyramidal cells. All these projections are excitatory and are rich in NMDA receptors, showing more immunoreactive cells in the perforant path and the Schaeffer collateral pathway (Bramham, Torp, Zhang, Storm-Mathisen, & Ottersen, 1990). CA1 in particular is the central nervous system region with the highest NMDA receptor density (Monaghan & Cotman, 1985).

In general, hippocampal plasticity, and LTP in particular, is linked to NMDA receptor stimulation (Cotman, Monaghan, & Ganong, 1987). Blockade of NMDA receptors has been shown to suppress LTP induction (Harris, Ganong, & Cotman, 1984). This correlates with studies that show that the blockade of NMDA receptors impairs hippocampal-dependent spatial learning (Morris, Anderson, Lynch, & Baudry, 1986). NMDA receptors play a role in several phases of LTP.

Synaptic strengthening, at least as it occurs in LTP, goes through a series of stages. According to Brown et al. (1986), LTP can be understood as a process that comprises three stages: induction, expression, and maintenance. Induction requires simultaneous processes at both the presynaptic and postsynaptic neurons. Specifically, glutamate released by the presynaptic neuron binds to postsynaptic NMDA receptors. This binding is possible when the postsynaptic membrane is sufficiently depolarized in a way that the magnesium ion that blocks an NMDA channel is liberated (Herron, Lester, Coan, & Collinridge, 1985; Thomson, 1986). Once the magnesium ion is released, calcium ions enter through the channel. The depolarization of the postsynaptic cell is due to induced stimulation that usually mimics a particular type of high frequency rhythm, theta, which in the hippocampal formation normally occurs during exploratory activity (Christian & Deadwyler, 1986; Green, McNaughton, & Barnes, 1990; Whishaw & Vanderwolf, 1983). When induced experimentally in a hippocampal pathway through a stimulating electrode, a tetanus represents bursts of four shocks of 100 Hz given at 200 ms intervals (Larson, Wong, & Lynch, 1986).

After potentiation has been induced, it is expressed through a series of mechanisms that mostly involve the activation of protein kinases. Calcium activates the production of kinases such as calcium-calmodulin kinase type II (CaMKII) and protein kinase C (PKC) (Akers, Lovinger, Colley, Linden, & Routtenberg,1986; Malenka et al., 1989; Malinow, Madison, & Tsien, 1988). These kinases act as second messengers that allow the neuron to be activated without the need of subsequent massive calcium influx, effectively converting the synapse into a potentiated one. These kinases however are important for the expression, but not the long-term duration or maintenance of the potentiation, at least at the presynaptic level (Malinow, Schulman, & Tsien, 1989).

The duration of the potentiation is due to a third process, namely maintenance. Although this remains one of the least understood mechanisms regulating LTP, it is known that potentiation entails a facilitation in the release of glutamate by the presynaptic neuron (Bliss, 1990; Bliss, Errington, Laroche, & Lynch, 1987; Bliss, Errington, & Lynch, 1990; Malgaroli et al., 1995; Malinow & Tsien, 1990). Protein synthesis also occurs in the entorhinal cortex after potentiation in the perforant path-dentate gyrus pathway, which might lead to long-term structural changes (see Kelly, Mullany, & Lynch, 2000). Structural changes occur as well as vesicle proteinic changes occur at the presynaptic level, at least in the dentate gyrus, after potentiation (Lynch, Voss, Rodríguez, & Bliss, 1994). Maintenance therefore seems to at least require long-term changes in the presynaptic neuron. However, changes have also been seen at the postsynaptic level. In general, after LTP induction, structural changes occur in the dendritic spines of cells in the three main hippocampal pathways (Yuste & Bonhoeffer, 2001). Structural changes have been observed in the length, size and density of dendritic spines (Muller, Nikonenko, Jourdain, & Alberi, 2002; Muller, Toni, & Buchs, 2000). Some of these changes have been related to changes in synaptic functions.

The interaction that occurs between neurons after LTP is established has diverse characteristics. Bliss & Collinridge (1993) identify three properties of LTP that define this interaction. These properties are cooperativity, associativity, and input-specificity. First, LTP occurs through the activation of not one but several inputs. The strength of potentiation is directly related to the number of presynaptic inputs. The integration of postsynaptic potentials produced by neurons in a pathway allows the postsynaptic neuron to be depolarized to a level at which the magnesium ion is released, terminating the blockade of

the NMDA channel. A second characteristic, associativity, refers to the temporal aspect of induction: several presynaptic cells should be active simultaneously in order for an activation threshold to be reached. In associativity different pathways that give input to the postsynaptic neurons can be activated and even the input can come from neurons activated by neurotransmitters other than glutamate. Even when one of the pathways is not strong enough to induce LTP (i.e., a weak input), it can become potentiated if the overall activity or summation of all the inputs produces LTP (see Barrionuevo & Brown, 1983). Finally, input-specificity refers to the fact that only those synapses of a pathway that were active during a tetanus become potentiated. Therefore, induction of a tetanus is unlikely to potentiate all synapses. This is important if LTP is to be used as a model for understanding memory formation since, hypothetically speaking, the synaptic changes that occur during learning of any specific item of information should entail changes in a subset of the neuronal connections (and not in all connections) within a pathway, region, or structure.

Several studies have assessed the relationship between LTP and learning. Since no single approach can provide a solid explanation of whether LTP is a model of memory formation, various methodological approaches that provide converging evidence are needed to sustain a coherent argument. In an evaluation of the LTP-learning/memory hypothesis, Martin, Grimwoood and Morris (2000) delineated five experimental strategies that have been used as basis for the assessment of the hypothesis. The five approaches are correlation, induction, occlusion, intervention, and erasure. These approaches will be examined briefly.

The correlation approach has been widely used and requires the comparison of performance in a learning task with the attempt to induce LTP. In one of the first studies using this strategy, Barnes (1979) implanted adult and senescent rats with both stimulating

and recording electrodes in the perforant path-dentate gyrus pathway, trained them on a spatial learning task, and later attempted to induce LTP. Compared to adults, senescent rats were impaired on the spatial task. After this, all rats were given high frequency stimulation three times and the rate of LTP decay was observed. LTP in senescent rats decayed faster than in adults, which correlated with the behavioral results.

Although correlational strategies are suggestive they do not address the issue of causation. Martin et al. (2000) considered a second approach, that of induction or "behavioral LTP" (Teyler & DiScenna, 1987). This perhaps might be one of the most compelling strategies because changes in synaptic plasticity are observed as a consequence of learning. If the synaptic changes observed during learning are similar to those observed during LTP, then the mechanisms that underlie LTP induction might explain how learning occurs at the cellular-molecular level. However, this strategy presents the problem that it is very difficult to determine the exact populations of neurons that are involved in the learning of specific information. Because both learning and LTP entail changes in specific populations of neurons, it is difficult to know beforehand where to look for changes. Therefore, a practical problem (i.e., where to record) poses limits to the use of this strategy.

A third approach examined by Martin and colleagues (2000) is that of occlusion, also known as saturation. In this strategy learning is assessed after a particular hippocampal pathway is potentiated multiple times to the point where further LTP cannot be induced. This would involve repeated high frequency stimulation at different sites within a pathway, after which further hippocampal-dependent learning would not be possible. The rationale would be that if the recording of experience requires the same type of synaptic change as LTP, then no learning can occur because of the impossibility of further synaptic change.

In a series of experiments, McNaughton and colleagues (McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986) saturated the perforant path to dentate gyrus cells of rats with multiple stimulations and assessed the animals' behavior in a circular platform for escape. Saturation had no behavioral effect on already learned information or on spatial working memory, but impaired the retention of recently acquired information and the learning of new spatial information. According to this, hippocampal synaptic change would be necessary for the learning of spatial information. The occlusion of further synaptic change through saturation hinders the ability to record experience. However, hippocampal synaptic change does not seem important for the retrieval of information from memory, nor does it seem necessary for the processing of spatial information or for navigation in general. The effects of occlusion therefore seem to be very specific. Later, McNaughton and colleagues (Castro, Silbert, McNaughton, & Barnes, 1989) observed that rats were able to learn a water maze spatial task at the same time that potentiation (or as they called it, enhancement) decayed. The strategy of occlusion consequently might give support to the hypothesis that the synaptic changes that occur during LTP are the same as those that record experience. The strategy has produced mixed results, which might be due to the difficulty in saturating the minimum number of fibers needed to occlude learning (see Martin et al., 2000). To determine that minimum is a technical challenge not likely to be solved in the near future.

A fourth approach discussed by Martin and colleagues (2000) is that of pharmacological and genetic interventions. In general, such interventions should impair hippocampus-dependent learning at the same time as they prevent LTP induction. Most of these interventions either manipulate NMDA receptors or some point in the cascade of events involved in maintenance.

The first type of intervention employed was pharmacological, using NMDA receptors antagonists. One of the first studies of this kind showed that intraventricular microinjections of the NMDA antagonist AP5 impaired the learning of a spatial but not a cued task in the water maze, while at the same time blocking LTP induction in the perforant path-dentate gyrus synapses (Morris, Anderson, Lynch, & Baudry, 1986). Studies like this provide strong support for the relationship between LTP and learning.

A more recent approach, genetic intervention, depends on gene deletion or in transgenesis (see Silva, 2003). The genetic intervention in this case would affect a specific aspect of the cascade of events necessary for LTP, while at the same time selectively impairing spatial learning in the absence of perceptual or motor impairments. The first of these attempts produced knockout mice for alpha-CaMKII who were deficient in both spatial learning on a water maze (Silva, Paylor, Wehner, & Tonegawa, 1992) and in vitro LTP induction in the Schaeffer collateral pathway to CA1 (Silva, Stevens, Tonegawa, & Wang, 1992).

The present author and colleagues (Nalbantoglu et al., 1997) studied transgenic mice expressing the amyloidogenic carboxy-terminal 104 amino acids of the amyloid precursor protein. The study correlated three parameters: learning of a spatial and a cued task, beta-amyloid immunoreactivity, and LTP induction in area CA1. For the behavioral study both young and aged control and transgenic mice were trained in visible and hidden tasks in the water maze. Transgenic mice were impaired in searching for the hidden, but not the visible platform, showing a selective deficit in spatial learning. Age exacerbated the deficit substantially in both young and aged mice. Transgenic mice showed an age-dependent increase in extracellular beta-amyloid immunoreactivity, increased gliosis and microglial reactivity, as well as cell loss in the CA1 region of the hippocampus. The behavioral and

histological analyses were consistent with an impairment of maintenance of LTP in CA1. In this case LTP was affected apparently due to a general disruption in cellular processing.

Other studies have generated mutant mice that allow the study of specific aspects of synaptic change and learning. For example, mutant mice for gene Zif268 present diminished overall LTP in dentate gyrus and faster decay rates, while at the same time are impaired in spatial learning (Bozon, Davis, & Laroche, 2002; Jones et al., 2001). Mice with an overexpression of cabining D(28k) (a calcium binding protein necessary for neuron excitability) in the dentate gyrus, also show reduced LTP and are impaired in spatial navigation (Dumas, Powers, Tarapore, & Sapolsky, 2004).

In general, genetic manipulations are an excellent strategy for correlating LTP and learning since they provide specific interventions at the molecular level that allow investigation of different steps in both learning and synaptic plasticity (see Hedou & Mansuy, 2003; Kandel, 2001; Silva, 2003). However, some genetic manipulations have produced deficits in LTP while not producing deficits in learning. For example, Brakebusch and colleagues (2002) found that knockout mice for the brevican gene suffered fast LTP decay in CA1 in the absence of motor behaviors, general exploration, and spatial learning in a water maze (brevican is expressed in neural tissue in the embryo and apparently regulates synaptogenesis).

More recently the intervention approach has combined two traditional interventions: pharmacology in rats and genetic manipulation of the mouse genome (see Chapman, 2002, for the latter). Drugs can be administered at an exact time so that the genetic expression is induced or blocked for a limited amount of time (see Higgins et al., 2002, and Ohno, Frankland, & Silva, 2002, for recent examples of the application of this technique). Using

this strategy mice are developed with a mutation that does not cause any deficit on itself. Then to assess learning and LTP induction, a drug is given at a subthreshold dose, but in an amount enough to cause a deficit. This allows study of the molecular level of learning and synaptic plasticity in mice that undergo normal development (i.e., similar to that of wild type mice), avoiding developmental deficits that can compromise the neural systems under study, if not the actual survival of the animals.

One final approach involves altering or erasing LTP *after* it has been established (Martin et al., 2000). The strategy consists of either repeatedly inducing high frequency stimulation or administering a drug that interrupts cellular processes a few minutes after LTP induction. The strategy does not seem to work once LTP is established. This would convert the strategy in one that intervenes directly in the processes needed for the expression of LTP, but not its maintenance. This approach has not been widely explored, nor there is evidence that frequent stimulation that erases LTP is able to abolish a learned behavior in an animal.

An interesting proposition by Martin and colleagues (2000) is an approach that would produce specific changes in learned behaviors *after* LTP is induced. If LTP in any way is similar to the synaptic functions that underlie memory, then the observed changes in synaptic function after potentiation would entail long-lasting changes in behavior. One study in this direction shows that LTP changes the activity of place cells (Dragoi, Harris, & Buzsaki, 2003). After potentiation, some established place fields disappeared while new ones were created. Overall, studies like this would help in understanding the relationship between synaptic activity and the coding of experiences.

All the approaches discussed address the question of whether LTP is a plausible model of memory formation in the hippocampal system. So far, the evidence seems to show

that research on LTP helps in understanding some of the mechanisms involved in the type of synaptic plasticity required for memory formation. However, it is important to stress two caveats. First, there is no evidence that suggests that LTP explains the totality of the mechanisms involved in memory formation. The neural recording of experience might easily prove to be a far more complex process than that researched so far through LTP. Ignoring momentarily the whole subjective process of the registering of experience and focusing only on its biological aspects, there might be far too many other interactions among structures, as well as many cellular-molecular processes that might contribute to memory formation, so that that LTP might prove to be a partial explanation. And this points toward a second caveat. Even when LTP might prove to be only a partial explanation, it is not even known whether the exact mechanisms required for LTP are the same as those necessary for hippocampal-related memory formation. Therefore, LTP as a model of memory formation might not only prove to be only a partial explanation, it might prove to be simplistic or sketchy at best. More research is needed to elucidate these questions.

D. Plan of experiments

To assess the role of NMDA receptors in rats' spatial learning and synaptic plasticity several experiments employing the use of NMDA antagonists were performed. At the same time the experiments were done as a mean to assess the adequacy of some of the models of hippocampal dependent learning presented in this chapter in an explanation of the role of hippocampal system NMDA receptors in learning.

In Chapter 2, I studied the induction of primed burst potentiation (PBP) in the perforant path-dentate gyrus pathway of freely moving male Long Evans rats after the systemic injection of NMDA antagonists. PBP, a form of synaptic plasticity similar to LTP

except in its durantion, is obtained after high frequency stimulation of hippocampal areas. The effect of the antagonists MK-801 and NPC 17442, a non-competitive and a competitive NMDA receptor antagonist respectively, was studied through dose-response curves. MK-801 was chosen since it has been used to study spatial learning in our as well in other labs. NPC 17442 was used here more extensively to establish its role in learning and synaptic plasticity. The dose-response curves established in this Chapter served as a basis for the pharmacological manipulations done later in behavioral studies reported in Chapters 3, 4, and 5.

In Chapter 3 I studied rats' spatial learning in a water maze task. Several doses of each NPC 17742 and atropine sulfate, a muscarinic cholinergic antagonist known to impair spatial learning, were administered to rats solving a cued and then a spatial task. The dose-response curves permited to establish low doses for each drug that were then administered in combination in a third experiment. The experiments had the purpose of evaluating the effects of NPC 17742 in spatial learning and studying the effects of a low dose of the drug when given together with a muscarinic receptor antagonist. Since both glutamate and acetylcholine are present in the hippocampal system, the experiments explored possible interactions between these neurotransmitters in the mediation of spatial learning.

The experiments reported in Chapter 4 served to study spatial learning and spatial working memory of rats administered NPC 17742 and trained in an 8/8 strategy in a radial arm maze. The studies had the purpose of investigating the effects of the antagonist in learning driven by a qualitatively different motivational value than that used in Chapter 3. While learning in the water maze is based on an aversive component, the radial maze task relies on appetitive learning. Each learning strategy may affect the interactions of the

hippocampal system with other brain systems for each task in different ways. The experiments also evaluated whether spatial learning and working memory could be dissociated at the level of NMDA receptors by blocking them with the competitive antagonist. While the hippocampal system is involved in spatial working memory, the role of NMDA receptors in this behavior is less clear. These experiments followed previous work with the non-competitive antagonist MK-801 (Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992).

In Chapter 5, a series of environmental and pharmacological manipulations was performed to further investigate the environmental dimensions of spatial learning. Through structured manipulations of the testing environments, I explored which aspects of the environment are relevant for the performance of an 8/8 strategy in a radial maze. Then, pharmacological manipulations tested learning and performance in well-trained rats. The study was done to explore the types of computations that animals perform for solving a spatial task. The results of these studies were discussed in terms of the implications they have for the prevalent view of spatial learning as the construction of a topographical map of the environment and the role that NMDA receptors may have in these processes.

CHAPTER 2. NMDA receptor antagonists MK-801 and NPC 17742 impair primed burst potentiation in the dentate gyrus of freely moving rats in a dose-dependent manner

INTRODUCTION

The representation of new information in the brain requires some form of plasticity, which is very likely to be found in the synapses between individual neurons. One form of synaptic plasticity that may encode and store spatial information is long-term potentiation (LTP; Lynch, 1986; McNaughton & Morris, 1987). LTP is easily produced in the hippocampus of rats (Barnes, 1988) and requires the binding of glutamate to the NMDA receptors for induction. Evidence for the involvement of LTP in learning and memory includes the observation that LTP is induced by spatial learning (Green & Greenough, 1986) and that the saturation of hippocampal synapses through repeated LTP prevents further spatial learning (McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986).

One of the hippocampal system structures where LTP has been studied is the dentate gyrus (see Bliss & Gardner-Medwin, 1973, and Bliss & Lømo, 1973, for the first LTP induction studies). Studies with neuropsychological patients and animals show the relevance of the structure in declarative learning. In most Alzheimer's disease patients, who during the early stages of the disease suffer from anterograde amnesia, there is severe damage to the entorhinal cortex-dentate gyrus pathway, mostly to cells containing glutamate receptors (Hyman et al., 1984, 1986, 1987; Lassmann, Fischer, & Jellinger, 1993). This prevents the hippocampus from receiving incoming cortical information.

Rodent studies show that within the hippocampal circuit, the dentate gyrus seems to be specially relevant for processing spatial information. Initial reports show a link between manipulations of the structure and performance on spatial learning tasks. For example, sub-

seizure electrical stimulation to the structure in rats immediately after training on a win-stay radial-arm maze task impairs subsequent learning (Collier & Routtenberg, 1983). Moreover, removal of dentate gyrus granule cells impairs spatial learning in a water maze task (Whishaw, 1987). Similarly, removal of granule cells either before or after training in an eight-arm radial maze with all or four baited arms impairs performance (McLamb, Mundy, & Tilson, 1988).

Further evidence for dentate gyrus involvement in spatial learning comes from developmental studies. First, it has been observed in newborn rats that food deprivation reduces neurogenesis in the dentate gyrus and mildly impairs learning in the spatial version of the water maze (Akman, Zhao, Liu, & Holmes, 2004). It has also been observed that levels of normal neurogenesis in the granule cell layer of the dentate gyrus are directly related to proficiency in the learning of a water maze task, with proficient rats presenting higher levels of dentate neurogenesis than less proficient rats (Drapeau et al., 2003). Normal aging has also been associated with loss of dentate gyrus synapses and spatial memory impairments. Old rats that show an impairment in solving a radial-arm maze task present a loss of axospinous synapses in the dentate gyrus, compared to both young and old rats that learned the same task (Geinisman, de Toledo-Morrell, & Morrell, 1986). Smith, Adams, Gallagher, Morrison, and Rapp (2000) obtained similar results, observing a high correlation between decreases in the molecular layer of the dentate gyrus of synaptophysin, a presynaptic vesicle glycoprotein that serves as a marker for synaptic density, and impairments in the spatial learning version of a water maze task. Together, these studies show that the dentate gyrus is relevant for spatial learning and for performance in working memory tasks that include a spatial component.

The dentate gyrus does not necessarily process all types of declarative learning, but seems to be specialized for learning specific types of information. Rats trained to choose a reward based either on the distance between two identical objects or on the order in which they had to enter the arms of a radial-arm maze, and that later received either dentate gyrus or CA1 lesions, were differentially impaired (Gilbert, Kesner, & Lee, 2001). Specifically, rats with lesions to the dentate gyrus were impaired at solving the task that was dependent on the distance between two objects, while they presented no impairment in the task that was based on temporal information. On the other hand, CA1 lesioned rats presented the reverse pattern of deficits. Therefore, the dentate gyrus seems to process space and not temporal order.

Another characteristic of dentate gyrus processing is that it seems to be relevant not for the acquisition of new information about individual stimuli, but for the the organization of stimuli in relation to one another, regardless of whether these are familiar or not. Jenkins and colleagues (Jenkins, Amin, Pearce, Brown, & Aggleton, 2004) observed expression of c-fos in a learning task involving spatial processing, but not in one where learning depended on encoding new information independently of its location. The expression of the gene was observed in the dorsal dentate gyrus, as well as in the rostral CA1 and CA3, after the rearrangement of the spatial configuration of familiar visual stimuli. However, c-fos expression was not observed after exposure to individual novel objects. Thus the dentate gyrus seems to be part of a circuit involved in the encoding of a novel spatial arrangement, and not necessarily in the encoding of discrete novel objects. The fact that c-fos expression is not affected by the degree of familiarity with objects suggests that the crucial information learned is the novel relationship between objects and not the objects per se.

Further evidence for the involvement of the dentate gyrus in the learning of novel places comes from Lee, Hunsaker and Kesner (2005). They posit that the dentate gyrus is involved in the detection of spatial novelty, but that it is not critical for the learning of novel objects. Rats lesioned in either the dentate gyrus or CA3 spontaneously explored a new object that was moved to a familiar location, but failed to explore familiar objects placed in novel locations. CA1 lesioned rats however presented mild deficits in the exploration of novel spaces that contained familiar objects. According to the authors, there seems to be a functional specialization in areas of the hippocampus, where the dentate gyrus is critical for novelty detection.

Changes in dentate gyrus synaptic plasticity associated with both learning and LTP induction are mediated by glutamate activation of NMDA receptors. For example, glutamate release is increased in the dentate gyrus after both training in the spatial version of the water maze in a novel environment (Richter-Levin, Canevari, & Bliss, 1995) and LTP induction within the same structure (Bliss, Errington, Laroche, & Lynch, 1987; Bliss, Errington, & Lynch, 1990). Moreover, NMDA antagonists that have been shown to impair spatial learning also impair LTP induction. For instance, NMDA antagonists d-aminophosphonovalerate (APV) and MK-801 have been shown to impair LTP induction in the dentate gyrus of anesthetized rats (Errington, Lynch, & Bliss, 1987; Richter-Levin et al.). Furthermore, intraventricular administration of d-2-amino-5-phosphonopentanoate (D-AP5), a competitive NMDA antagonist, at a concentration that blocks the induction of hippocampal LTP, impairs spatial learning in the water maze (Davis, Butcher, & Morris, 1992). Thus, glutamate activation of NMDA receptors in the dentate gyrus seems to be necessary for both LTP and spatial learning.

The experiments reported in this chapter measure synaptic plasticity in the perforant path-dentate gyrus pathway of freely moving rats injected with NMDA antagonists employing a type of lasting synaptic strenghtening called primed burst potentiation (PBP). PBP is induced by primed burst stimulation (PBS), a pattern that resembles some aspects of the rhythmic neuronal activity known as theta (see Diamond, Dunwiddie, & Rose, 1988). Theta is a rhythmic EEG oscillation of 4-12 Hz observed in the granule cells of the dentate gyrus during exploratory behaviors (Buzsáki, 1989; Rose, Diamond, & Lynch, 1983). Diamond and colleagues observed that the use of frequencies outside those ocurring during theta rhythm failed to induce PBP, suggesting that the synaptic changes observed after PBS are due to hippocampal cell activity that is similar to that observed during exploration. For PBP induction a burst must be delivered optimally within a period between 140 and 170 ms following a leading pulse, which would represent 6-7 Hz (Rose & Dunwiddie, 1986).

PBP has been induced in CA1 in both hippocampal slices and freely moving rats, and is impaired in slice preparations by administration of the competitive NMDA antagonist AP5 or the noncompetitive NMDA antagonist PCP (Diamond, Dunwiddie, & Rose; Kentros et al., 1998; Rose & Dunwiddie, 1986). Here, through the use of dose-response curves, we wanted to establish the minimal dose of noncompetitive NMDA antagonist MK-801 and competitive NMDA antagonist NPC 17742 that would impair the establishment of dentate gyrus PBP of behaving rats 30 min after PBS. The doses selected for each drug were also used in behavioral studies assessing spatial learning to explore the relationship between NMDA mediated synaptic plasticity and spatial learning. These studies are reported in Chapters 3, 4 and 5.

Noncompetitive and competitive NMDA antagonists have distinct mechanisms of action, producing different effects within the same type of receptor. A widely studied noncompetitive NMDA antagonist is MK-801 {10,11-dihydro-5-methyl-5H-dibenzo [a,d] cycloheptene-5,10 imine}. Originally synthesized as an anticonvulsant, it has a high affinity and selectivity for NMDA receptors, with the hippocampus being the brain structure with the highest density for binding (Wong et al., 1986). The drug antagonizes the effects of glutamate on NMDA (but not on non-NMDA) receptors by binding to the cation channel within the receptor, preventing calcium influx (Woodruff et al., 1987; Wong, Knight, & Woodruff, 1988). The drug has temporary effects that are not noticeable at least one day after administration. Whishaw and Auer (1989) reported the dissapearance of the drug effects on EEG and behavior when both parameters were re-evaluated five days after injection. Similarly, Ward, Mason and Abraham (1990) reported finding no effect on spatial learning when rats were injected with the drug 24 hr before training. When injected within 1 hr before testing however, effects have been observed both on physiological responses and learning. Specifically, the drug has been shown to impair PBP induction in the dentate gyrus of female Sprague Dawley rats (Hargreaves, Côté, & Shapiro, 1997) at a dose that affects place but not cued learning in the radial arm maze in rats of the same sex and strain (Caramanos & Shapiro, 1994; Shapiro & O'Connor, 1992). Initial unpublished observations by the author using male Long-Evans rats in behavioral studies however suggested possible sex and strain differences compared to the results of Shapiro and colleagues. Partly to explore these sex and strain differences in terms of the MK-801 dose, we conducted a PBP study using a dose-response curve.

A less studied NMDA antagonist is NPC 17742 [2R,4R,5S-2-amino-4,5-(1,2-cyclohexyl)-7-phosphoheptanoic acid; Ferkany et al., 1993; Willetts et al., 1993]. Since the drug is a competitive antagonist, it has a different mechanism of action than MK-801, acting directly at the glutamate binding site, preventing the neurotransmitter from acting on the receptor unless the presence of the neurotransmitter is increased above the typical levels normally found in the cell population. Previous studies showed that NPC 17742 prevents LTP induction in the dentate gyrus of anesthetized male Long Evans rats (Hetherington, Austin, & Shapiro, 1994). Unpublished observations by the present author showed that the dose used in that study, 10 mg/kg, produced locomotor impairments in rats of the same strain and sex (e.g., rats were ataxic, fell from arms of a radial maze, were not be able to stand on a platform, or drooled excessively). Because of this, we explored whether lower doses would still have an effect on synpatic plasticity –in this case PBP instead of LTP. Also, since we wanted to use the same dose that impaired PBP also in behavioral studies, we decided to do electrophysiological recordings in freely moving animals. This would override the effects that anesthesia has on the levels of currents that would have to be used to obtain PBP (see Engstrom et al., 1990), while at the same time would allow for a more naturalistic observation, of course, within the constrained conditions imposed by an experimental setting in a controlled environment. For the study we tested two doses to then later explore a relationship between dose and the extent of behavioral impairment in the water maze (see Chapter 3) and radial-arm maze (Chapter 4).

The purpose of the experiment, therefore, was to establish dose-response curves for the effect of both drugs on PBP. This information formed the basis for their use in

subsequent behavioral studies that assessed the role of NMDA mediated plasticity in spatial learning and performance.

METHOD

The method described here is a variation of the the one published in Hargreaves et al. (1997).

Subjects

Eight Long-Evans rats (Charles Rivers, St. Constant, Québec) were used. The rats weighed 300-400 g at the start of the experiment and were housed individually in transparent cages, in a temperature-controlled room with a light cycle from 7 am to 7 pm. All animals had ad libitum access to food and water.

Recording apparatus

All recording took place within a cylindrical Plexiglas recording chamber (30 cm diameter; 40 cm tall) located inside of a standard wood and metallic mesh Faraday cage (72 X 90 X 90 cm). Stimulating and recording electrodes were twisted, bipolar wires (127 μm diameter), with tips vertically separated by 0.5 mm, constructed of Teflon coated stainless steel, and soldered to male gold-plated pins (Amphenol 220-P02 Relia-Tac pin). The pins were assembled into McIntyre miniature connectors (STC-89P1-220, Carleton University, Ottawa, Ontario). The connectors were embedded in a head-stage made of dental acrylic, connected to matching female pins (Amphenol 220-502 Relia-Tac socket), and assembled into one plastic socket (STC-89S1-220) locked by a ring nut (STC-89N1-220). A series of lightweight shielded wires was soldered to the female pins and attached to a commutator (Josef Biela Engineering, Irvine, CA), which was mounted on top of the Faraday cage. Individual shielded cables continued from the commutator to a differential AC amplifier (A-

M Systems, model No.1700, Bellvue, WA). Output from the amplifier was digitized and recorded with an AT-386 PC, and analyzed with the Common Processing module software (DataWave Systems, Longview, CO). Stimulation pulses were generated by an analog stimulus isolator unit (A-M Systems, SIU model No.2200, Bellvue, WA). The stimulation parameters were determined by the computer.

Surgery

Rats were surgically implanted with one recording and one stimulating electrode. Prior to surgery, each rat was given atropine sulfate (2.5 mg/kg, sc) to prevent respiratory problems, and anesthetized with a combination of diazepam (5 mg/kg, sc) and ketamine (100 mg/kg, im). After an appropriate anesthetic level was reached, the rat was placed in a stereotaxic apparatus, hair over the skull was clipped, the scalp was incised along the longitudinal plane and retracted, and the skull was positioned in a horizontal plane. Connective tissue was cleared and holes were drilled for screw and electrodes placement. Then, the rat was placed within the Faraday cage for lowering both electrodes. The recording and stimulating electrodes were lowered slowly and independently from one another over the course of several minutes to prevent tissue damage, and implanted in the granular/hilar region of the dentate gyrus (AP: – 3.6, ML: ± 1.8, DV: – 3.9; Paxinos & Watson, 1986) and in the ipsilateral perforant path (AP: – 8.0, ML: ± 4.4, DV: – 3.1; Paxinos & Watson), respectively. Screws serving as ground and recording indifferent were embedded in the frontal and interparietal skull plates. During lowering of the electrodes, the final position within a horizontal plane was determined by physiological responses obtained with the recording setup. In this procedure the final dorsoventral position of the recording electrode is determined by delivering stimuli and observing the evoked response. When the

stimulating and recording electrodes are in the appropriate positions, the evoked potentials (EPs) including excitatory postsynaptic potentials (EPSPs) and population spikes (PS) have a stereotyped shape and can be adjusted to maximize these parameters. Animals that exhibited epileptiform activity or after discharge during the placement of electrodes were discarded from the study. Once an appropriate response was obtained, the electrodes were cemented in place with dental acrylic and the male pins were assembled into the connector plug. The plug was then anchored to the skull and screws using dental acrylic. After the dentaly acrylic dried, rats were taken from the stereotaxic instrument, given 100 μl Tribrissen antibiotic (sc, Coopers Agropharm Inc., Ajax, Ontario), and kept on a heating pad until they recovered from anesthetic, when they were then returned to their home cages. The antibiotic was administered again 24 hr later. Rats were given a minimum of 7 days to recover from surgery. Thereafter, animals were monitored for infections and secure head-stage placements. Surgeries were performed following CCAC guidelines.

Drug treatment

For each recording session rats were injected intraperitoneally with one of the following six treatments: saline; 0.0625, 0.08, or 0.25 mg/kg of MK-801; 3 or 5 mg/kg of NPC 17742. All rats received all treatments during separate sessions. A randomized design for the schedule of the treatments was implemented.

Procedure

Several sessions were given to habituate the rats to the procedures, and to monitor the stability and placement of the recording electrodes. After these habituation sessions, recording sessions followed, each animal receiving not more than one session within a period of at least 3 days.

For each session, cables were connected gently to the head-stage connector plug, secured with the ring nut, and the rat was placed in the clear cylindrical recording chamber centered in the Faraday cage. Recording commenced within 1 min after cables were connected to the connector plug. Rats were left to freely explore the recording environment.

EPs were generated by stimulating the perforant path with diphasic, bipolar pulses (0.1 ms per phase), delivered at a frequency no higher than 0.1 Hz. They were recorded at a sampling rate of 20 KHz. For each EP the amplitude of a negative-going PS measure was collected. The amplitude was measured as the distance going from a tangent marking the onset and offset of the PS to the maximal negative-going peak of the PS.

EPs were collected by delivering test pulses generated with five pre-selected current intensities (ranging from 200 to 1000 μA) to determine an input/output (I/O) curve. For each animal the intensities were selected from the minimal one needed to produce a noticeable PS, to the minimal one needed to produce the largest PS amplitude for that particular rat. The three other intensities produced PSs of an amplitude of roughly 25, 50 and 75 % of the one produced using a pulse of the highest intesity. For each I/O curve, EPs were produced by delivering test pulses in ascending order of intensity every 15 s, for I/O curves of an approximate duration of 13 min.

A total of four I/O curves were collected during a recording session. The first two curves were collected before PBS, at 20 and 47 min, and allowed to measure stability of the EP (by comparing the two curves) and a pre-PBS baseline (the second curve) against which post-PBS responses would be compared, respectively. The two other I/O curves were collected after PBS (which was delivered at 60 min after injection): one after 45 s and the other after 30 min. These last two curves measured the immediate effects of PBS (first post-

PBS curve) and stability or any possible decay that had ocurred 30 min after PBS (second post-PBS curve). Whenever I/O curves were not collected, test pulses were delivered every 15 s using the third (i.e., medium) intensity level to visually monitor stability of the EP.

PBS was delivered 60 min after injection during behavioral immobility. PBS consisted of a 1000 μA constant stimulation current, given by a leading pulse followed at an interval of 170 ms by a burst of 200 Hz. Pulses making up the primed burst were otherwise identical to test pulses. Post-PBS levels were monitored until about 43 min after PBS. Individual EPs were analyzed offline for further statistical analyses.

Histology

At the end of the experiments, rats were given an overdose of chloral hydrate and the electrode tip placements were marked by passing 30 μA DC through the tips for 3 s. Rats were then perfused intracardially with normal saline followed by a 10 % formaldehyde solution / 30 % sucrose prussian blue (potassium ferricyanide) solution. Coronal sections 60 μm thick were cut on a freezing microtome and stained with cresyl violet to highlight cell layers and verify electrode positioning. All rats with acceptable recordings had placements located in the granular/hilar region of the dentate gyrus.

Statistical analysis

The second pre-PBS I/O curve, or pre-PBS baseline, was transformed as a 100 % value of the average of all EPs collected during that I/O curve, following Kentros et al. (1998) method for comparing pre- and post-PBS levels. The other three I/O curves (corresponding each to the periods of 20 min after injection, right after PBS, and 30 min after PBS) are presented as a percentage of the baseline level. The data were analyzed by repeated

measures analysis of variance (ANOVA). I/O curves for each rat were used as factors in the analysis.

RESULTS

Induction of PBP in control sessions

All rats used in the experiments showed PBP under control conditions (see Figures 2.1-2.2). Comparison of the pre-PBS baseline and the 13 min period right after PBS shows that the perforant path-dentate gyrus pathway became potentiated: $F(1, 14) = 35.32$, $p < .001$. PBP remained robust at least until 43 min after induction as shown by comparison between the pre-PBS baseline period and the second post-PBS period: $F(1, 14) = 16.16$, $p < .001$. Comparisons between the first and second post-PBS periods showed that potentiation did not seem to decay during the period recorded after PBP induction: $F(1, 14) = 0.004$, $p = .95$. Lastly, granular cell responses evoked by perforant path stimulation remained stable before PBP induction. Comparisons of the two pre-PBS periods did not show differences in cellular responses: $F(1, 14) = 2.31$, $p = .15$.

Effects of MK-801 on the induction of PBP

Experiments using three doses of MK-801 showed that the drug impaired PBP induction in a dose dependent manner. While the 0.0625 mg/kg dose did not have a noticeable effect, both the 0.08 and 0.25 mg/kg doses effectively impaired potentiation.

The 0.0625 mg/kg dose failed to block PBP induction as shown by comparisons between the pre-PBS baseline level and the first post-PBS period: repeated measures ANOVA, effect of time, $F(1, 14) = 15.96$, $p < 001$ (see Figure 2.1, panel A). The PBP induced in the rats that received 0.0625 mg/kg was as strong as in saline controls: repeated measures ANOVA, effect of groups, $F(1, 14) = 0.14$, $p = .71$. Potentiation remained robust

until the end of the period recorded after PBP induction, showing no signs of decay: repeated measures ANOVA, effect of time, $F(1, 14) = 1.08$, $p = .32$. Saline and 0.0625 mg/kg rats did not differ either in rate of decay of potentiation, with PBP remaining robust in similar ways in both groups: repeated measures ANOVA, effect of groups, $F(1, 14) = 1.84$, $p = .2$.

The 0.8 mg/kg dose did not block the induction of potentiation, but caused a subsequent decay (see Figure 2.1, panel B). During the initial post-PBS period, there was an increase in PS amplitude compared to pre-PBS baseline: repeated measures ANOVA, effect of time, $F(1, 14) = 50.26$, $p < .001$. The magnitude of potentiation did not vary between the drug and saline groups: repeated measures ANOVA, effect of group, $F(1, 14) = 2.86$, $p = .11$. However, potentiation significantly decayed in the treatment group 30 min after induction. Comparisons between the first and second post-PBS periods showed a decrease in PS amplitude: repeated measures ANOVA, effect of time, $F(1, 14) = 15.8$, $p < .001$. Potentiation levels in drug treated rats differed from that of saline controls 30 min after PBS: repeated measures ANOVA, effect of group, $F(1, 14) = 8.42$, $p < .05$.

The 0.25 mg/kg dose significantly affected the induction of potentiation (see Figure 2.1, panel C). Initially, a significant increase in the PS amplitude was attained during the 5 min immediately after PBS. Comparisons between those 5 min and the 5 min prior to PBS (baseline levels) show a brief duration increase in response: repeated measures ANOVA, effect of time, $F(1, 14) = 11.26$. $p < 01$. However, the increase in PS amplitude decayed after 5 min, as measured by the second half of the initial post-PBS period against pre-PBP baseline: repeated measures ANOVA, effect of time, $F(1, 14) = 3.77$, $p = .07$. During the second post-PBS period the PS amplitude returned to pre-PBS baseline levels: repeated

measures ANOVA, effect of time, $F(1, 14) = 2.18$, $p = .16$. Saline and drug treated rats did not differ in potentiation levels during the period immediately after PBS, but they did 30 min after, with potentiation decaying in the 0.25 mg/kg group: repeated measures ANOVA, effect of group, $F(1, 14) = 10.13$, $p < .01$.

Finally, MK-801 did not seem to alter EPs across time prior to PBS, as measured by the amplitude of PSs obtained during the first and second pre-PBS periods (see Figure 2.1). For the the 0.065 mg/kg dose: repeated measures ANOVA, effect of time, $F(1, 14) = 1.5$, $p = .24$. For the 0.08 mg/kg dose: repeated measures ANOVA, effect of time, $F(1, 14) = 1.89$, $p = .19$. For the 0.25 mg/kg dose: repeated measures ANOVA, effect of time, $F(1, 14) = 1.3$, $p = .27$.

Effects of NPC 17742 on the induction of PBP

Experiments using two doses of NPC 17742 showed that the drug affected the events after PBS in a dose dependent manner. While the 3 mg/kg dose did not have an effect, the 5 mg/kg dose produced a decay in PBP (see Figure 2.2).

The 3 mg/kg dose failed to block PBP induction as shown by comparisons between the pre-PBS baseline level and the first post-PBS period: repeated measures ANOVA, effect of time, $F(1, 14) = 52.04$, $p < 001$ (see Figure 2.2, panel A). PBP was induced in 3 mg/kg rats as strong as in saline controls: repeated measures ANOVA, effect of groups, $F(1, 14) = 0.87$, $p = .37$. Potentiation remained robust at least 43 min after PBS, showing no signs of decay: repeated measures ANOVA, effect of time, $F(1, 14) = 1.33$, $p = .27$. Saline and 3 mg/kg rats did not differ either in rate of decay of potentiation, with PBP remaining robust in similar ways in both groups until the end of the recording sessions: repeated measures ANOVA, effect of groups, $F(1, 14) = 2.01$, $p = .18$.

The 5 mg/kg dose prevented potentiation (see Figure 2.2, panel B). Although, saline and drug treated rats did not differ significantly during the period immediately after potentiation [repeated measures ANOVA, effect of groups, $F(1, 13) = 2.89$, $p = .11$], comparisons of EPs between this period and pre-PBS baseline in the drug treated group showed no significant differences: repeated measures ANOVA, effect of time, $F(1, 12) = 3.85$, $p = .07$. Thirty minutes after PBS, the PS amplitude significantly decayed in the treatment group, with responses returning closer to pre-PBS baseline: repeated measures ANOVA, effect of time, pre-PBS baseline vs. second post-PBS period, $F(1, 12) = 4.07$, $p = .07$. For this second post-PBS period, saline and drug treated rats significantly differed: repeated measures ANOVA, effect of groups, $F(1, 13) = 5.3$, $p < .05$.

Similar to MK-801, NPC 17742 did not seem to alter EPs across time during the baseline period (see Figure 2.2). No significant differences were observed in between amplitude of PSs obtained during the first and second pre-PBS periods for any of the doses: 3 mg/kg, repeated measures ANOVA, effect of time, $F(1, 14) = 0.13$, $p = .73$; 5 mg/kg: repeated measures ANOVA, effect of time, $F(1, 12) = 2.88$, $p = .12$.

DISCUSSION

In this study, PBP in the perforant path-dentate gyrus pathway of freely moving male Long Evans rats was impaired by injections of either of two NMDA antagonists, MK-801 and NPC 17742, in a dose dependent manner. The same rats showed an increase of about 50 % in PS amplitude when treated with saline. In both drug conditions however, while a significant increase in PS amplitude was attained at all doses during the 5 min immediately after PBS, this effect later disappeared, with higher doses producing faster rates of decay.

Establishing dose-response curves was necessary for several reasons. Some NMDA antagonists have effects on other neurotransmitter systems (see Löscher, Annies, & Hönack, 1991, for effects of MK-801 on dopaminergic and serotonergic neurotransmission). Some NMDA antagonists may also produce behavioral side effects (e.g., Hargreaves & Cain, 1992; Hegberg & Rose, 1989; Löscher & Hönack, 1992). Because of this, it was desirable to establish the lowest effective dose for each drug that impaired PBP for correlation with behavioral studies assessing spatial learning in Chapters 3, 4, and 5. The results of the present study show that decreases in potentiation have a direct relation with increases in doses. Therefore, 0.08 mg/kg of MK-801 and 5 mg/kg of NPC 17742 were set as adecuate minimal doses to establish a correlation with spatial learning tasks dependent on hippocampal integrity.

In this study, we found that MK-801 impaired PBP induction only at doses higher than the one of 0.0625 mg/kg used before in female Sprague Dawley rats by Hargreaves et al. (1997) to attenuate PBP induction. The dose used by Hargreaves and colleagues had previously been found to impair spatial learning of a radial-arm maze task in rats of the same strain and sex (Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). Because we used male Long Evans rats in the studies presented in subsequent chapters, we wanted to establish a dose that would impair PBP in animals of this particular strain and sex. In unpublished observations using Long Evans rats, the author found that higher doses of MK-801 were needed for attaining learning deficits comparable to those obtained with Sprague Dawley female rats. Previously, there have been some reports on sex differences in the use of this drug. The first such reports were on opioid-mediated analgesia after MK-801 injections in mice (Lipa & Kavaliers, 1990). Later, it was found that locomotion was impaired in male

Wistar rats using much higher doses of MK-801 than in females, suggesting that there could be a sex difference in the modulation of NMDA receptors (Hönack & Löscher, 1993). Strain differences have also been found in reactivity to MK-801 (e.g., Oliff, Marek, Miyazaki, & Weber, 1996). Finally, in electrophysiological studies of hippocampal synaptic plasticity, identical protocols for the induction of long-term depression produce disparate results in different strains of freely-moving rats (Manahan-Vaughan, 2000). These studies and the results presented here suggest that the effects of NMDA antagonists on synaptic transmission may vary according to strain and sex. In terms of methodology, this should be taken into consideration when planning studies or comparing data on the effects of NMDA antagonists that employ rats of different sexes and strain.

Regarding NPC 17742, this is the first report of impairments in PBP induction after the administration of the drug. Previously it had been shown that LTP induction could be blocked in the dentate gyrus by injections of 10 mg/kg of the drug in urethane anesthetized male Long Evans rats (Hetherington et al., 1994). Here we observed that changes in synaptic plasticity can be prevented with half that dose. This is important to avoid effects of the drug in locomotion. Unpublished observations by the author showed that doses higher than 5 mg/kg cause severe locomotor impairments. This is another reason why it was desirable to find the lowest effective dose that impaired synaptic plasticity.

Contrary to LTP, PBP is a form of short-term plasticity that does not last more than a day. From a methodological standpoint this might be advantageous. Unlike in LTP, potentiation can be induced multiple times, allowing for within subjects comparisons. In LTP studies, even in a control treatment, sometimes potentiation can not be induced due to incorrect electrode placement. Since LTP can be induced only once, it is sometimes difficult

to ascertain that the lack of potentiation is due to the effects of the drug or to electrode placement. Although not frequent, even after histological verification apparently "well-implanted" electrodes might fail to produce LTP. In PBP studies where multiple treatments are given to the same subject across time, rats that do not show potentiation under control conditions can be excluded from the study. Those that fail to show PBP under drug but not control conditions are included in the analysis, reducing variance since different treatments are exerted upon the same physiological system.

In the present study, the fact that NMDA antagonists affected the rate of decay of PBP but not its initial induction can be interpreted in terms of the mechanisms of plasticity it affects. Similar results have been observed in LTP studies on CA1 slices treated with PKC inhibitor polymyxin B, in which potentiation is initially induced with the PS and EPSP returning to baseline levels 10 min after tetanic stimulation (Reymann, Frey, Jork, & Matthies, 1988). This form of short-term potentiation can be attributed to non-NMDA mechanisms like the activation of group I metabotropic glutamate receptors (mGluRs). These receptors, of which there is a high density in the rat dentate gyrus (especially of the type 1; Lavreysen, Pereira, Leysen, Langlois, & Lesage, 2004), mediate the induction and maintenance of LTP in that structure and CA1 (Aiba et al., 1994; Balschun et al., 1999; Bashir et al., 1993; Behnisch & Reymann, 1993; Bordi, 1996; Bordi, Reggiani, & Conquet, 1997; Lapointe et al., 2004; Naie & Manahan-Vaughan, 2005; Perez, Morin, & Lacaille, 2001). In this study, it is possible that the short-term increase in PS amplitude observed immediately after PBS occured through the activation of mGluRs. The activation of group I mGluRs after high-frequency stimulation promotes the liberation of calcium from intracellular calcium stores (ICSs; Manahan-Vaughan, Braunewell, & Reymann, 1998;

Wilsch, Behnisch, Jäger, Reymann, & Balschun, 1998). Weak forms of high-frequency stimulation liberate calcium from ICSs, causing a transient increase in intracellular calcium levels, facilitating potentiation (see Balschun et al.). This is consistent with studies that show that agonists of group I mGluRs facilitate LTP in the dentate gyrus if given 5 min immediately after tetanization, with this facilitation being less effective if subsequent administrations are given at later intervals (Manahan-Vaughan & Reymann, 1996). The antagonists used in this study most likely affect the calcium influx through the NMDA channels that normally is produced after PBS. mGluR activation however might cause a momentary increase in calcium that affects the EP for a short period of time. Since the observed increase in potentiation is short lived, NMDA antagonists might effectively block the long-term plastic changes associated with high frequency stimulation and presumably learning.

The study of PBP might contribute to the understanding of the relationship between declarative learning and hippocampal plasticity in a manner similar to that of LTP (see Chapter 1 for a discussion), with the exception that the temporary nature of PBP mimics the temporary nature of synaptic changes observed in the dentate gyrus after learning. Manipulation of NMDA receptors through the use of competitive and noncompetitive antagonists could help in the understanding of the synaptic changes that occur during learning and PBP. If the same dose that affects PBP induction in the dentate gyrus of freely moving rats also selectively affects spatial learning, this would suggest that this type of learning might be subserved by the types of synaptic changes observed during PBP.

Figure 2.1. Graphs show the effects of primed burst potentiation (PBP) in the granular region of the dentate gyrus after primed burst stimulation (PBS) to the ipsilateral perforant path in rats treated with either saline or one of three doses of noncompetitive NMDA antagonist MK-801. Each graph shows four recorded time periods where the current used for regular stimulation produced about 50 % of the maximum response that could be obtained. Data are normalized to the average baseline values prior to PBS 60 min after injection (time of PBS is marked as 0 min). In saline treated rats (n = 8), potentiation remained stable 43 min after PBS, as measured by an increase in the population spike (PS) amplitude of 50-52 % (average for each post-PBS time period) above pre-PBS baseline. MK-801 impaired PBP in a dose dependent manner in the granular region of the dentate gyrus. The lowest dose, 0.0625 mg/kg (n = 8) failed to produce any significant change in the PS amplitude (Panel A). Neither of the higher doses prevented immediate PBP induction. With 0.08 mg/kg (n = 8) PBP decayed over 30 min after PBS (panel B). With 0.25 mg/kg (n = 8) PBP decayed within 5 min (panel C).

Figure 2.1

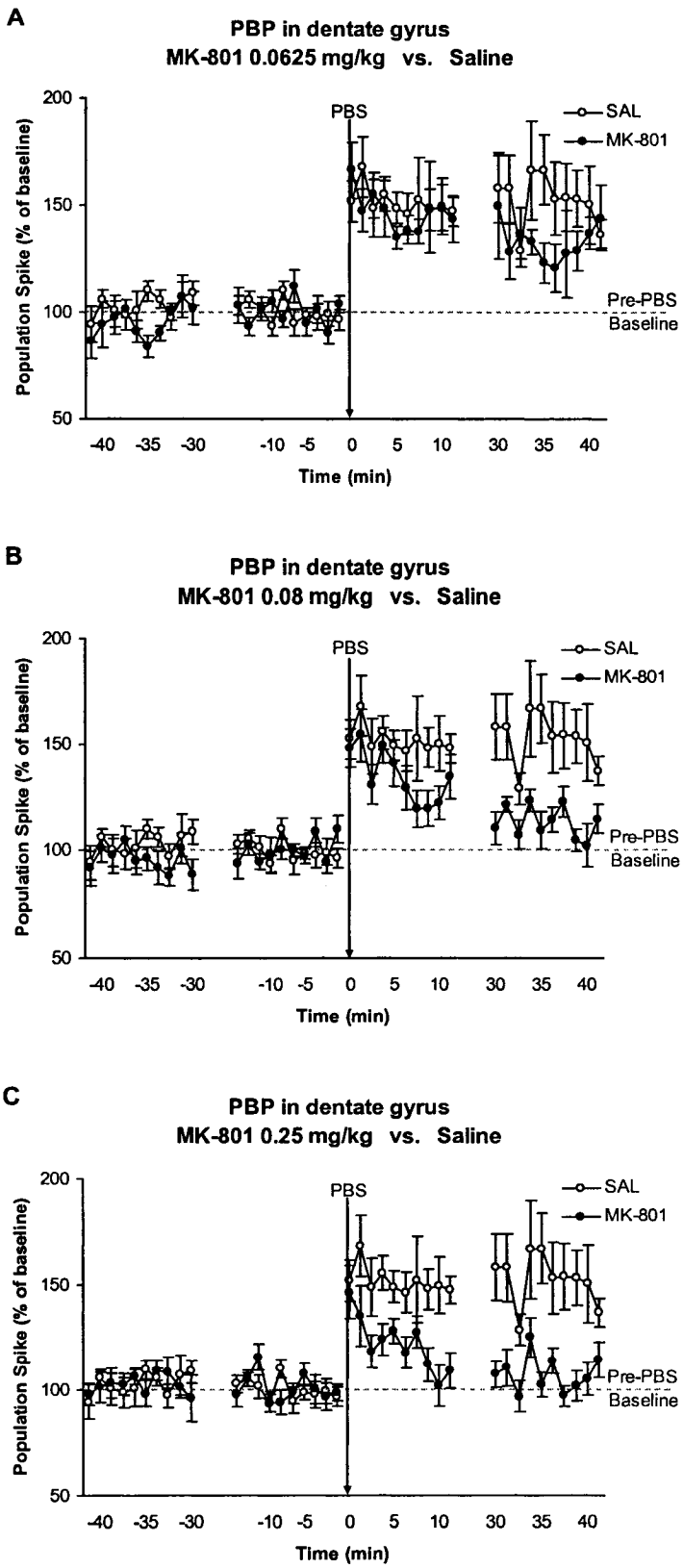
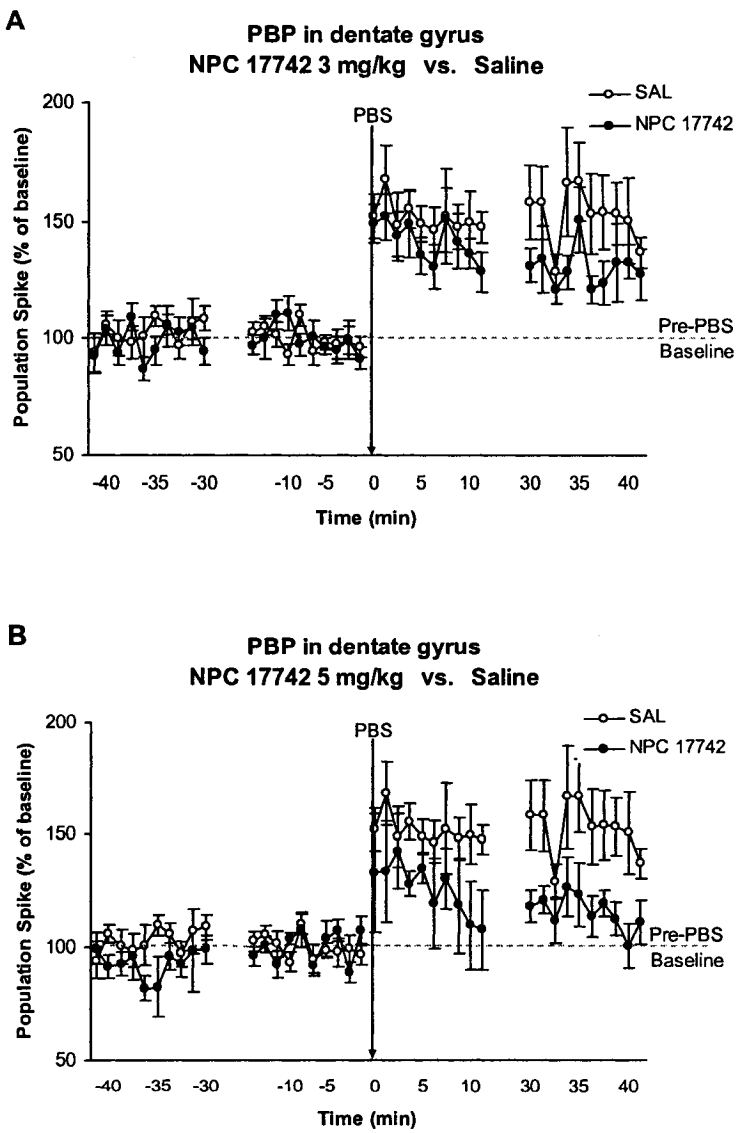

A
PBP in dentate gyrus
MK-801 0.0625 mg/kg vs. Saline
PBS
SAL
MK-801
Pre-PBS Baseline
Population Spike (% of baseline)
200
150
100
50
-40 -35 -30 -10 -5 0 5 10 30 35 40
Time (min)
B
PBP in dentate gyrus
MK-801 0.08 mg/kg vs. Saline
PBS
SAL
MK-801
Pre-PBS Baseline
Population Spike (% of baseline)
200
150
100
50
-40 -35 -30 -10 -5 0 5 10 30 35 40
Time (min)
C
PBP in dentate gyrus
MK-801 0.25 mg/kg vs. Saline
PBS
SAL
MK-801
Pre-PBS Baseline
Population Spike (% of baseline)
200
150
100
50
-40 -35 -30 -10 -5 0 5 10 30 35 40
Time (min)

Figure 2.2. Graphs show the effects of primed burst potentiation (PBP) in the granular region of the dentate gyrus after primed burst stimulation (PBS) to the ipsilateral perforant path in rats treated with either saline or one of two doses of competitive NMDA antagonist NPC 17742. Each graph shows four recorded time periods where the current used for regular stimulation produced about 50 % of the maximum response that could be obtained. Data are normalized to the average baseline values prior to PBS 60 min after injection (time of PBS is marked as 0 min). In saline treated rats (n = 8), potentiation remained stable 43 min after PBS, as measured by an increase in the population spike (PS) amplitude of 50-52 % (average for each post-PBS time period) above pre-PBS baseline. NPC 17742 impaired PBP in a dose dependent manner. The lowest dose of NPC 17742, 3 mg/kg (n = 8), failed to block PBP or cause PBP decay in the granular region of the dentate gyrus (Panel A). The highest dose, 5 mg/kg (n = 7), impaired PBP induction causing a decay of PS amplitude 30 min after PBS (Panel B).

Figure 2.2


A
PBP in dentate gyrus
NPC 17742 3 mg/kg vs. Saline
SAL
NPC 17742
PBS
Pre-PBS
Baseline
Population Spike (% of baseline)
200
150
100
50
-40 -35 -30 -10 -5 0 5 10 30 35 40
Time (min)
B
PBP in dentate gyrus
NPC 17742 5 mg/kg vs. Saline
SAL
NPC 17742
PBS
Pre-PBS
Baseline
Population Spike (% of baseline)
200
150
100
50
-40 -35 -30 -10 -5 0 5 10 30 35 40
Time (min)

CHAPTER 3. The role of NMDA and muscarinic receptors in spatial navigation: Effects of the combined administration of NPC 17742 and atropine on the learning of spatial and cued versions of a water maze task

INTRODUCTION

Glutamate (Baudry & Lynch, 1981; Butelman, 1989; Caramanos & Shapiro, 1994; Richter-Levin, Canevari, & Bliss, 1995; Fagg & Foster, 1983; Riedel, Platt, & Micheau, 2003; Shapiro, 2001; Shapiro & O'Connor, 1992; Shapiro & Caramanos, 1990; Storm-Mathisen, 1981) and acetylcholine (Costa, Panula, Thompson, & Cheney, 1983; Fibiger, 1991; Rogers & Kesner, 2003; Sutherland, Whishaw, & Regehr, 1982; Whishaw, 1985, 1989) are present in many projections to the hippocampal system and are involved in hippocampus-mediated spatial learning and memory. These facts suggest that the two systems may act synergistically in the manifestation of spatial learning and memory. In this study, this possibility was explored in the water maze in rats administered NMDA and muscarinic receptor antagonists given both individually and in combination.

Glutamatergic and cholinergic depletion are two of the main characteristics of Alzheimer's disease (AD) neurodegeneration. AD entails a progressive decline in most higher cognitive functions including spatial and more general declarative learning impairments (Hyman, Damasio, Damasio, & Van Hoesen, 1989; Hyman, Van Hoesen, & Damasio, 1990). The neurodegeneration that occurs in AD has various manifestation at the cellular level (i.e, neurofribrillary tangles, neuritic plaques, Lewy and Hirano bodies; see Hyman, Damasio, Damasio, & Van Hoesen, 1989). However a common factor in most AD patients is the loss of cells and general neurodegeneration in the hippocampus or its connections (Ball et al., 1985; Hyman, Van Hoesen, Damasio, & Barnes, 1984). This could be associated with the anterograde amnesia observed in the early stages of the disease.

Initially, the cognitive disorders observed in AD patients were attributed to the degeneration of cholinergic fibers (Bartus, Dean, Beer, & Lippa, 1982; Coyle, Price, & DeLong, 1983; Whitehouse et al., 1982), but more recent research focused on glutamatergic depletion, especially in the enthorinal cortex afferents to the hippocampus (Hyman, Van Hoesen, & Damasio, 1987; Hyman, Van Hoesen, Kromer, & Damasio, 1986). AD-associated neuropathology and studies that show that antagonists of NMDA receptors can disrupt spatial learning, (Morris, Anderson, Lynch, & Baudry, 1986; Shapiro & Caramanos, 1990) led to the speculation that in humans glutamate in the hippocampal system is necessary for declarative learning. The possibility of a cooperative contribution from both neurotransmitters to spatial learning is not fully understood.

Glutamate and learning

Glutamate is expressed in cortex and the limbic system (Cooper, Bloom, & Roth, 2002). The hypothesis that depletion of the neurotransmitter in the hippocampal system contributes to the learning impairments of AD is supported by that fact that there is a decrease in the level of free glutamate in the perforant pathway of AD patients (Hyman et al., 1987). Further support for this notion includes a marked decrease in glutamate levels in the temporal cortex of AD patients (Procter et al., 1988), a positive correlation between metabolite levels in the cerebrospinal fluid of AD patients and their scores in cognitive tests (Smith, Bowen, Francis, Snowden, & Neary, 1985), and abnormal NMDA receptor-mediated excitotoxicity (Olney, Wozniak, & Farber, 1997). However, the relationship between glutamatergic depletion and learning impairments in AD is not clear because it remains unknown whether glutamatergic dysfunction is a prerequisite for dementia. (see Palmer & Gershon, 1990).

The role of glutamate in learning and memory is studied more directly through pharmacological agents that block NMDA receptors. NMDA antagonists AP5 and MK-801

impair spatial learning in both the radial arm maze (Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992; Caramanos & Shapiro, 1994; Parada-Turska & Turski, 1990) and the water maze (Morris et al., 1986). The water maze requires escape to a platform that is slightly submerged in a pool filled with opaque water (Morris, 1981). While the radial arm maze depends on searching for a positive reward (i.e., food), navigation in the the water maze entails the escape from an aversive situation. Both tasks permit measuring behavior in different ways. The radial arm maze allows to differentiate between reference and working memory. The water maze permits the assessment of two qualitatively distinct types of learning, spatial and cued, that roughly employ the same motor strategies. On the cued task, animals must learn a stimulus-response association, which usually depends on the integrity of the basal ganglia (Packard & McGaugh, 1992). On the spatial task, a hidden platform must be found in the pool which presumably requires the construction of a map of the environment. Administration of D-AP5 impairs place learning (Davis, Butcher, & Morris, 1992; Morris, Anderson, Lynch, & Baudry, 1986) but not place recall (Butcher, Hendry, & Morris, 1989; Heale & Harley, 1990) in this task. This suggests that NMDA receptors are important for the acquisition of the task but not for performance. The administration of MK-801 impairs spatial but not cued learning (Robinson, Crooks, Shinkman, & Gallagher, 1989; Whishaw & Auer, 1989), which suggests that NMDA receptors are important for learning only the spatial version of the task.

Acetylcholine and learning

Acetylcholine in expressed in diverse areas of the nervous system. In the peripheral nervous system, acetylcholine is released at parasympathetic ganglionic synapses and at neuromuscular junctions (Gearien & Mede, 1974). In the mammalian central nervous system there are two major cholinergic pathways: the basal forebrain and the pontine (Cooper, Bloom, & Roth, 2002; Everitt & Robbins, 1997). The basal forebrain pathway includes the medial

septal nucleus, the diagonal band of Broca, the substantia innominata, the magnocellular preoptic field, and the nucleus basalis of Meynert. All of these basal forebrain structures contain cholinergic cell bodies and fibers that project to the cortex, the amygdala, and the hippocampus (McKinney, Coyle, & Hedreen, 1983; van der Zee & Luiten, 1999). The pontine pathway consists of the pedunculopontine and laterodorsal tegmental nuclei, the pontine and medullary reticular formations, the deep cerebellar and vestibular nuclei, and the cranial nerve nuclei (Cooper et al., 2002).

Human psychopharmacological studies implicate the cholinergic system in learning and memory, though the precise effects, actions, and mechanisms of these cholinergic drugs are not fully understood (see Schon et al., 2005, for recent use of fMRI to investigate the localized effects of anticholinergic drugs). Antimuscarinic drugs such as atropine sulfate and scopolamine can disrupt learning in a delayed match to sample task (Schon et al., 2005), encoding in fear conditioning tasks (Rogers & Kesner, 2004), and can produce transient amnesic states (Coyle, Price, & DeLong, 1983).

A role for acetylcholine in learning and memory is suggested by the degeneration during AD of brain structures related to learning and memory (see Fibiger, 1991). The neocortex and hippocampus of AD patients usually shows a substantial reduction in the activity of choline acetyltransferase (Palmer & Gershon, 1990). In addition, basal forebrain cholinergic neurons, mostly those in the nucleus basalis of Meynert and the medial septum, degenerate during the disease (Whitehouse et al., 1982). The spatial learning and memory deficits in AD patients may occur partly because of the deprivation of cholinergic input to the hippocampus.

Pharmacological interventions and lesion studies in rats provide further evidence for a role of the neurotransmitter in learning. Atropine sulfate, which blocks cholinergic muscarinic receptors, impairs the acquisition of a spatial task in the water maze (Hagan, Tweedie, & Morris, 1986; Sutherland, Whishaw, & Regehr, 1982; Whishaw, 1985, 1989; Whishaw & Tomie, 1987), but does not interfere with the acquisition of nonspatial tasks (Whishaw, 1985, 1989; Whishaw & Petrie, 1988; Whishaw & Tomie, 1987). Selective lesions to the magnocellular neurons of the basal forebrain, which provide the major cholinergic input to the cortex, affect incrementing but not decrementing attention to conditioned stimuli (Chiba, Bucci, Holland, & Gallagher, 1995). Studies have also combined lesions with pharmacological interventions to assess anatomical specificity. Septal lesions, combined septal and nucleus basalis lesions, but not nucleus basalis lesions produce moderate deficits in the spatial version of the water maze task (Nilsson & Gage, 1993). The administration of atropine sulfate exacerbates the deficits suggesting that the septohippocampal cholinergic system is closely implicated in spatial learning.

Acetylcholine in the hippocampus seems to be implicated in spatial learning and memory. Microdialysis studies in rats solving a radial arm maze task over a 12-day period show that increased acetylcholine release in the hippocampus correlates with better performance (Fadda, Cocco, & Stancampiano, 2000). In a single unit recording study, the administration of scopolamine in rats caused a displacement of previously recorded place fields (i.e, place cells fire in other, usually adjacent, locations after drug administration; Brazhnik, Muller, & Fox, 2003). According to the authors, the muscarinic blockade in this case seems to affect the firing of neurons necessary for processing a specific location.

The contribution of the cholinergic system to spatial learning is not clear. Rats pre-trained on a place task prior to drug treatment are not impaired in a new environment when administered atropine sulfate (Whishaw, 1985). Whishaw (1989) suggests that acetylcholine might be necessary for learning a navigational strategy that employs distal cues, but might not be necessary for learning spatial relations (see Whishaw, 1989).

Interactions between glutamate and acetylcholine: A possible role in spatial learning

The direct connections between the basal forebrain cholinergic system and the hippocampus through the septum, the presence of glutamate in every region of the hippocampus, the degeneration of brain structures associated with these neurotransmitters in AD, and the fact that antagonists for both neurotransmitters impair learning suggest that these two neurotransmitter systems may interact in learning and memory (see Aigner, 1995). The activation of muscarinic receptors, which are preferentially involved in learning (see van der Zee & Luiten, 1999), causes a rapid but transient increase in NMDA receptor activation in the hippocampal formation (Auerbach & Segal, 1996; Segal & Auerbach, 1997). Apparently, the activation of muscarinic receptors affects glutamate release, but has no direct effect in the postsynaptic binding of glutamate into NMDA receptors (Segal, 1989). The fact that place fields are displaced after scopalamine administration (Brazhnik et al., 2003) suggests the possibility that hippocampal glutamate and acetylcholine have roles in spatial learning and processing. Since place field stabilization is mediated by NMDA receptors (Kentros et al., 1998), it is possible that both glutamate and acetylcholine interact in the formation of a spatial representation.

Several behavioral studies show that concurrent administration of an antagonist of each neurotransmitter produces learning deficits in hippocampal dependent tasks. The administration of MK-801 exacerbates impairments produced by the administration of

scopolamine in a radial arm maze task (Li, Matsumoto, Tohda, Yamamoto, & Watanabe, 1996). Combined administration of both drugs impairs performance in an inhibitory avoidance learning task, which depends on place learning (Ohno & Watanabe, 1996). Similarly, the combined administration of MK-801 and scopolamine produces deficits in a delayed non-matching to sample task in monkeys (Matsuoka & Aigner, 1996). The administration of one antagonist without the other failed to have an effect, suggesting that an interaction between both neurotransmitter systems occurs during the learning of the task. Finally, the combined administration of subthreshold doses of scopalamine and MK-801 impairs performance in an elevated plus maze (Hliňák & Krejčí, 1998). In conclusion, the these drugs seem to have an additive effect that causes or exacerbates learning deficits.

The experiments reported here had two purposes. The first was to evaluate whether NPC 17742 had any effect in the learning of a water maze spatial task that was preceded by non-spatial pre-training. Consistent with the PBP results in Chapter 3, three doses were used: the minimal dose that impairs PBP, and two lower doses. The second aim of the study was to test whether the combination of low doses of NPC 17742 and atropine sulfate, (a drug that causes impairments in the water maze, Whishaw, 1985), has additive effects. An additive effect might suggest that both neurotransmitter systems interact in spatial learning.

A dose-response curve for each drug was established in separate experiments testing spatial and cued learning in the water maze. In a third experiment, the maximum dose for each drug that did not cause a behavioral impairment was used alone or in combination.

METHOD

Subjects

Ninety-six male Long-Evans rats (Charles Rivers, St. Constant, Québec) rats were used. The rats weighed 275-300 g and were three months of age at the start of the experiment. The rats were housed in pairs in transparent cages, in a temperature-controlled room with a light cycle from 7 am to 7 pm. All animals were trained daily between 9 am and 1 pm, and had ad libitum access to food and water.

Apparatus

Two circular pools were used to test for cued and spatial learning, in that order. Only one pool was used for each task, and each pool was located in a different room. This allowed to dissociate the learning of the swimming strategy towards a goal from the learning of the spatial attributes of a room.. Each pool was filled with water at 20-21 °C that was made opaque with paint (250 ml of non-toxic liquid tempera). For purposes of statistical analysis, the pool was divided into four quadrants as in a Cartesian plane, at the end of each experiment.

The pool used for cued learning measured 167 cm in diameter, 37 cm from the rim of the pool to the surface of the water, and was filled with water to a depth of 38 cm. The pool used for spatial learning measured 170 cm in diameter, 27 cm from the rim of the pool to the surface of the water, and was filled with water to a depth of 37 cm.

A solid platform was placed in each pool. The platform used for the cued task was constructed of wood and measured 13 X 13 cm and 5 cm tall. The sides of the platform were covered with a strip of black towel, and the top with a piece of white towel. The platform was raised 3-3.5 cm above water level. The platform used for the spatial task measured 10 X 10 cm and was constructed of Plexiglas. This platform was covered with a piece of white towel, and was 1.5-2 cm below water level.

A video camera, located above the pool, was connected to a video cassette recorder and computer which recorded the animal's position in the pool at all times.

The rooms that contained the two pools each had many salient extra-maze cues that included posters, lamps, and various three-dimensional objects. All of these cues, as well as the location of each pool, remained fixed throughout the experiment.

General Procedure

Handling. Prior to each experiment, each rat was handled 5 min per day, for four consecutive days.

Spatial and cued learning. After handling, each rat was trained to swim to a platform located in the center of a quadrant. Rats were trained for either three or four days (Experiment 1 and Experiments 2 and 3, respectively) on a cued task, followed by either six or eight days (Experiment 1 and Experiments 2 and 3, respectively) on a spatial task. During the cued task, the platform was placed in a new quadrant every day, but it remained in the same location throughout all training sessions for the spatial task. Before each daily session each rat was injected with an antagonist or vehicle. A daily session consisted of four trials. For each trial, a rat was placed in the water facing the wall at the edge of the pool, at one of the four starting points located at the rim of the pool, between the outer edges of each quadrant. The sequence of starting points was chosen randomly, so that each starting location was used once per day. Every rat was given a maximum of 30 s to find the platform. If a rat did not climb onto the platform before this maximum time period, it was guided to the platform. A 10 s rest period on the platform was given between trials. At the end of the training session the rat was dried with a towel and placed in a holding cage.

Probe trial. A single probe trial was given the day following the final day of spatial training. The probe trial was given in the room designated for spatial learning. The rats were not given injections prior to the probe trial, and two animals from every group were randomly assigned to one of the four starting locations. For the trial the platform was removed and each rat was placed in the water, in the manner decribed above, and allowed to swim for 60 s before being removed from the pool.

Procedure for Experiment 1: Atropine sulfate 3, 5 and 7 mg/kg

The subjects, apparatus, and procedure of this experiment were the same as those described above in "General Methods". All of the animals were randomly assigned to one of four groups (n = 8 per group): either saline or one of three doses of atropine sulfate (3, 5 or 7 mg/kg, i.p.) 20 minutes prior to training.

Procedure for Experiment 2: NPC 17742 3, 4 and 5 mg/kg

The subjects, apparatus, and procedure of this experiment were the same as those described above in "General Methods". The procedure for this experiment also included random assignment of the animals to one of four groups (n = 8 per group) that received either distilled water or one of three doses of NPC 17742 (3, 4, or 5 mg/kg, i.p.), 60-61 min prior to training.

Procedure for Experiment 3: Atropine sulfate and NPC 17742

The subjects, apparatus, and procedure of this experiment were the same as those described in "General Methods". For this experiment, all of the animals were randomly assigned to one of four groups (n = 8 per group): vehicle (n = 4 distilled water; n = 4 saline), 3 mg/kg of NPC 17742, 3 mg/kg of atropine sulfate, and both 3 mg/kg of NPC 17742 and 3

mg/kg of atropine sulfate. The NPC 17742 and the distilled water were administered 60-61 min prior to testing, and the atropine sulfate and the saline were administered 20 min prior to testing.

Statistical Analyses

Escape latency, (i.e., the time that each rat spent in the water before finding the platform) was used to assess the performance of rats during training. The daily individual averages across trials were calculated at the end of the last trial and were used in the final analysis. Probe trials were analyzed calculating the total time that each rat spent in each of the four quadrants.

The data were analyzed by repeated measures analysis of variance (ANOVA). The factors used in the analysis were groups and days.

RESULTS

Experiment 1: Atropine sulfate 3, 5 and 7 mg/kg dose-response curve

All animals learned the cued task (repeated measures ANOVA, effect of days, saline: $F(2, 14) = 37.88, p < .001$; 3 mg/kg: $F(2, 14) = 63.02, p<0.001$; 5 mg/kg: $F(2,14) = 16.29, p < .001$; 7 mg/kg: $F(2, 14) = 12.71, p < .002$. Furthermore, the rate of learning of the drug groups did not differ significantly from the control group (repeated measures ANOVA, effect of groups, 3 mg/kg vs. saline: $F(1, 14) = 0.11, p > .05$; 5mg/kg vs. saline: $F(1, 14) = 1.53, p > .05$; 7mg/kg vs. saline: $F(1, 14) = 3.22, p > .05$; see Figure 3.1).

All four groups were capable of acquiring the task (repeated measures ANOVA, effect of days, saline: $F(5, 35) = 23.91, p < .001$; 3 mg/kg: $F(5, 35) = 8.83, p < .001$; 5 mg/kg: $F(5, 35) = 14.17, p < .001$; 7 mg/kg: $F(5, 35) = 5.01, p < .002$. However, the 5 and 7 mg/kg groups were impaired, learning at a slower rate than the control group (repeated measures ANOVA, effect of

groups, 3 mg/kg vs. saline: $F(1, 14) = 0.16$, $p > .05$; 5 mg/kg vs. saline: $F(1, 14) = 7.02$, $p < .02$; 7 mg/kg vs. saline: $F(1, 14) = 35.79$, $p < .001$; see Figure 3.1).

On the probe trial, all groups showed a spatial bias for the target quadrant (repeated measures ANOVA, effect of quadrants, saline: $F(3, 21) = 30.27$, $p < .001$; 3 mg/kg: $F(3, 21) = 11.29$, $p < .001$; 5 mg/kg: $F(3, 21) = 8.89$, $p < .002$; 7 mg/kg: $F(3, 21) = 9.91$, $p < .001$). However, only the 3 and 5 mg/kg groups spent as much time as the control group in the target quadrant, with the 7 mg/kg group differing significantly from the control group (ANOVA, effect of groups, 3 mg/kg vs. saline: $F(1, 14) = 0.62$, $p > .05$; 5 mg/kg vs. saline: $F(1, 14) = 3.17$, $p > .05$; 7 mg/kg vs. saline: $F(1, 14) = 7.16$, $p < .02$; see Figure 3.2).

Experiment 2: NPC 17742 3, 4 and 5 mg/kg dose-response curve

All four groups of animals learned the cued task (repeated measures ANOVA, effect of days, control: $F(3, 21) = 24.74$, $p < .001$; 3 mg/kg: $F(3, 21) = 35.99$, $p < .001$; 4 mg/kg: $F(3, 21) = 14.28$, $p < .001$; 5 mg/kg: $F(3, 21) = 11.05$, $p < .001$; see Figure 3.3). The 5 mg/kg group of NPC 17742 did not learn the task as well as the control group (repeated measures ANOVA, effect of groups, 3 mg/kg vs. control: $F(1, 14) = 0.15$, $p > .05$; 4 mg/kg vs. control: $F(1, 14) = 2.32$, $p > .05$; 5 mg/kg vs. control: $F(1, 14) = 4.73$, $p < .05$).

All animals were capable of learning the spatial task (repeated measures ANOVA, effect of days, control: $F(7, 49) = 6.78$, $p < .001$; 3 mg/kg: $F(7, 49) = 10.94$, $p < .001$; 4 mg/kg: $F(7, 49) = 4.99$, $p < .001$; 5 mg/kg: $F(7, 49) = 5.97$, $p < .001$; see Figure 3.3). However, analysis for group differences showed that only the 3 mg/kg group did not differ from the control group (repeated measures ANOVA, effect of groups, 3 mg/kg vs. control: $F(1, 14) = 0.53$, $p > .05$; 4 mg/kg vs. control: $F(1, 14) = 11.86$, $p < .005$; 5 mg/kg vs. control: $F(1, 14) = 17.72$, $p < .002$). The swim latencies of the 4 mg/kg group however did not differ significantly

from the control group during the last half (four days) of spatial training, indicating that this group eventually reached a level of performance similar to the control group (repeated measures ANOVA for the last four days of spatial training, effect of groups, $F(1, 14) = 3.46$, $p > .05$). The 5 mg/kg group remained impaired across days.

On the probe trial, all groups showed a bias for the target quadrant (repeated measures ANOVA, effect of quadrants, control: $F(3, 21) = 38.36$, $p < .001$; 3 mg/kg: $F(3, 21) = 22.75$, $p < .001$; 4 mg/kg: $F(3, 21) = 24.32$, $p < .001$; 5 mg/kg: $F(3, 21) = 12.47$, $p < .001$; see Figure 3.4). The groups did not differ in the amount of time spent in the target quadrant (ANOVA for groups, 3 mg/kg vs. control: $F(1, 14) = .57$, $p > .05$; 4 mg/kg vs. control: $F(1, 14) = 0.53$, $p > .05$; 5 mg/kg vs. control: $F(1, 14) = 4.04$, $p > .05$). However, post hoc analysis of the least significant difference for the control and 5 mg/kg groups approached significance ($p = .054$), suggesting that the drug might have impaired learning of the location of the hidden platform.

Experiment 3: Atropine sulfate 3 mg/kg and NPC 17742 3 mg/kg

All groups of rats were capable of learning the cued task (repeated measures ANOVA, effect of days, control: $F(3, 21) = 48.96$, $p < .001$; NPC 17747: $F(3, 21) = 51.54$, $p < .001$; atropine sulfate: $F(3, 21) = 33.09$, $p < .001$; atropine sulfate and NPC 17742: $F(3, 21) = 19.60$, $p < .001$; see Figure 3.5). All rats learned the task at the same rate since there were no differences between groups for the task (repeated measures ANOVA, effect of groups, NPC 17742 vs. control $F(1, 14) = 4.19$, $p > .05$; atropine sulfate vs. control: $F(1,14) = 0.07$, $p > .05$; NPC 17742 and atropine sulfate vs. control: $F(1, 14) = 0.9$, $p > .05$).

All groups also acquired the spatial task (repeated measures ANOVA, effect of days, control: $F(7, 49) = 15.23$, $p < .001$; NPC 17742: $F(7, 49) = 12.47$, $p < .001$; atropine sulfate: $F(7, 49) = 21.17$, $p < .001$; atropine sulfate and NPC 17742: $F(7, 49) = 13.54$, $p < .001$).

However, the NPC 17742 group showed an impairment since it learned at a slower rate than the control group (repeated measures ANOVA, effect of groups, NPC 17742 vs. control: $F(1, 14) = 11.43$, $p < .005$; atropine sulfate vs. control: $F(1, 14) = 0.02$, $p > .05$; NPC 17742 and atropine sulfate vs. control: $F(1, 14) = 1.73$, $p > .05$; see Figure 3.5).

The latencies of rats administered 3 mg/kg of NPC 17742 and vehicle in Experiments 2 and 3 were combined for statistical analyses ($n = 16$ for each group) to obtain a global estimate of the effect of both treatments. The results for the combined data were similar to those of the non-combined data in Experiment 3 for both the cued and spatial tasks. Neither vehicle nor NPC 17742 impaired the cued task (repeated measures ANOVA, effect of groups: $F(1, 30) = 1.24$, $p > .05$). NPC 17742 impaired the learning of a spatial task (repeated measures ANOVA, effect of groups, $F(1, 30) = 6.07$, $p < .05$; see Figure 3.6).

On the probe trial, all groups showed a spatial bias for the target quadrant. Furthermore, all the drug treated groups spent approximately the same amount of time as the control group in the target quadrant. Therefore, all groups learned the location of the platform in the spatial task (repeated measures ANOVA, effect of quadrants, control: $F(7, 49) = 15.23$, $p < .001$; NPC 17742: $F(7, 49) = 12.47$, $p < .001$; atropine sulfate: $F(7, 49) = 21.17$, $p < .001$; NPC 17742 and atropine sulfate: $F(7, 49) = 13.54$, $p < .001$. There were no significant differences between groups in terms of preference for the target quadrant (ANOVA for groups: NPC 17742 vs. control: $F(1, 14) = 0.82$, $p > .05$; atropine vs. control: $F(1, 14) = 4.41$, $p > .05$; NPC 17742 and atropine vs. control: $F(1, 14) = 1.70$, $p > .05$; see Figure 3.7).

DISCUSSION

NPC 17742 impaired spatial but not cued learning in a dose-dependent manner. The results are consistent with the dose-dependent impairments in PBP (Chapter 2). The combined

administration of the drug with atropine sulfate did not have an effect. The results of this study suggest that the relationship between the glutamate and acetylcholine neurotransmitter systems in spatial learning in the water maze may not be additive.

In Experiment 1, determining a dose-response curve for atropine sulfate allowed to choose a maximum dose that did not produce impairments in spatial and cued learning in the water maze. Atropine impaired spatial but not cued learning in a dose-dependent manner. Doses higher than 3 mg/kg slowed the rate of spatial learning. Despite this, all groups showed a bias for the target quadrant. However, the 7 mg/kg group differed from the control group in terms of target quadrant preference. Because the 7 mg/kg group showed impairments in all spatial learning measures, and the 5 mg/kg group showed a mild deficit during training, 3 mg/kg was chosen for the third experiment.

In Experiment 2, the same method used in Experiment 1 served to choose an appropriate maximum dose that did not produce an impairment in either spatial or cued learning in the water maze. Animals given the lowest dose of NPC 17742 were capable of both spatial and cued learning in the water maze. The 4 and 5 mg/kg groups learned the spatial task at a slower rate than controls, with the higher dose differing significantly from controls throughout all sessions of spatial training. All groups showed a spatial bias on the probe trial for the target quadrant, indicating that they learned the location of the platform. The statistical analyses of the probe trial suggest that a difference between the 5 mg/kg and control group might arise if the number of sessions for spatial training is shortened or if the dose is slightly increased. The 3 mg/kg dose of NPC 17742 was chosen for the final experiment because animals given this dose learned and performed at the level of controls throughout all phases of training and testing.

The results in all NPC 17742 water maze experiments contrast with those of Saucier and Cain (1995). They found that 5 mg/kg of NPC 17742 did not impair spatial learning in the water maze in rats that had received non-spatial pretraining. They suggest that NMDA receptor antagonists might cause sensorimotor disturbances rather than spatial learning deficits. In Experiments 2 and 3 various doses of the drug produced deficits in the spatial task in rats that had already learned a cued task. Sensorimotor side effects were not observed during any of the tasks. The reasons for the differences in results are not clear. One possibility is that they are due to variations in the experimental protocols. In the study by Saucier and Cain, rats were given 10 trials to search for a hidden platform followed by 10 trials to search for a visible platform. It would be interesting to test whether there is a time window for the action of the drug, or whether the number of trials (i.e, four vs. 10 spatial plus 10 non-spatial) implies a different type of processing.

Experiment 3 suggests that the relationship between the acetylcholine and glutamate neurotransmitter systems in spatial learning in the water maze is not necessarily additive. Different from Experiment 2, in Experiment 3, 3 mg/kg of NPC 17742 produced an impairment. This difference might be attributed to secondary variables which might have significant effects when subthreshold doses are employed in different experiments. The combined data of both experiments though showed that there were significant differences between drug and control groups. NPC 17742 retarded spatial learning, but this was not observed when the drug was given in combination with 3 mg/kg of atropine sulfate. This dose of atropine sulfate alone did not impair spatial or cued learning. Therefore, one possibility is that atropine attenuated the impairments produced by the NMDA antagonist during spatial training.

Atropine has been shown to enhance glutamate transmission by binding to inhibitory muscarinic receptors located in glutamate terminals of the hippocampus. The binding of acetylcholine to these receptors in rat hippocampal synaptosomes prevents the release of glutamate (Marchi & Raiteri, 1989). The administration of atropine supresses the inhibitory action of acetylcholine in the inhibitory muscarinic receptors (Marchi, Bocchieri, Garbarino, & Raiteri, 1989). The administration of a muscarinic antagonist thus might result in an enhancement in glutamate release in the hippocampus. Since an NMDA antagonist at a low dose will only cause a partial blockade of receptors, the supression of the activity of inhibitory muscarinic receptors results in an enhancement of glutamate neurotransmission. This could constitute a compensatory mechanism that allows learning to occur.

It is possible that the relationship between glutamate and acetylcholine varies during specific behaviors and across brain regions. In some situations, learning may require the activation of one neurotransmitter system and a decrease in neurotransmission in another. Hasselmo and Bower (1993) propose that acetylcholine may have neuromodulatory roles during learning and remembering. According to the model, a decrease of cholinergic transmission is necessary for suppressing the recall of stored information, allowing thus the learning of new material by reducing interference. Cholinergic suppression of excitatory and inhibitory afferents has been observed in the olfactory cortex and in CA1 and CA3, where acetylcholine decreases transmission through a presynaptic mechanism (see Hasselmo & Bower; Valentino & Dingledine, 1981).

Although both glutamate and acetylcholine are relevant for learning, each one mediates behavior differently. In Hasselmo and Bower's (1993) model, acetylcholine in the hippocampus serves as a neuromodulator of presumably glutamate neurotransmission. Some

behavioral experiments show that the learning deficits caused by NMDA and muscarinic receptor antagonists are not always equivalent, suggesting that each neurotransmitter system contributes uniquely to the mechanisms that support learning. The administration of either scopolamine or MK-801 decreases accuracy in a repeated sequence acquisition task (Cohn, Ziriax, Cox, & Cory-Slechta, 1992). However, while scopolamine produces rats to skip portions of the learned sequence, MK-801 induces rats to repeatedly choose the first two choices. In monkeys performing a computerized delayed non-matching to sample task each drug also increased errors, but in different manners (Ogrura & Aigner, 1993). Scopolamine increased response latency, while MK-801 increased choice inaccuracy. Other studies using a delayed matching to position task (Stanhope, McLenachan, & Dourish, 1995) and delayed non-matching to position task (Robinson & Mao, 1997) have also found distinct behavioral effects for each type of drug. In conclusion, although both muscarinic and NMDA receptor antagonists affect learning, they do so by altering different learning processes.

The results from this study show that the relationship between glutamate and acetylcholine is not always additive. These two neurotransmitter systems do not show redundancy in function and might contribute to different aspects of the learning of a water maze task. The results also are consistent with the hypothesis that NMDA receptors are necessary for the learning of a spatial information.

Figure 3.1. Graphs show the average latencies per day for cued and spatial training of rats given saline or atropine sulfate (3, 5 or 7 mg/kg, $n = 8$ for each group). Panel A. shows all groups. Panels B, C, and D show the saline group compared with the 3, 5 and 7 mg/kg groups, respectivey. All rats were able to learn the cued task. Only rats given 7 mg/kg of atropine sulfate were impaired in the spatial task.

Figure 3.1

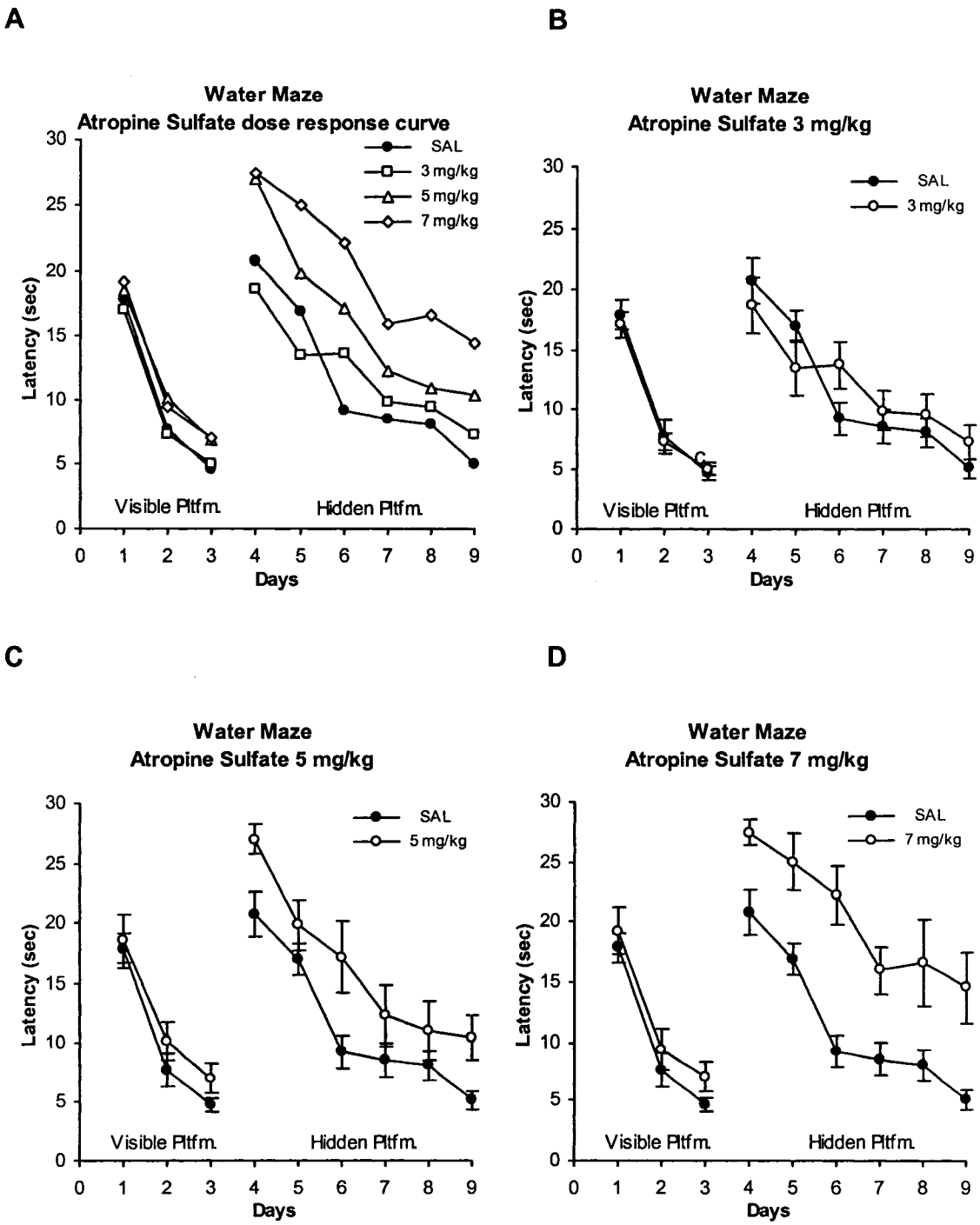

A
Water Maze
Atropine Sulfate dose response curve
SAL
3 mg/kg
5 mg/kg
7 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
B
Water Maze
Atropine Sulfate 3 mg/kg
SAL
3 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
C
Water Maze
Atropine Sulfate 5 mg/kg
SAL
5 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
D
Water Maze
Atropine Sulfate 7 mg/kg
SAL
7 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days

Figure 3.2. Graphs show time spent in a quadrant for each group: saline (panel A), and 3 (panel B), 5 (panel C), or 7 mg/kg (panel D) of atropine sulfate. All groups spent more time in the target quadrant than in the others during the 60 s probe trial. Each bar shows a quadrant. The target quadrant is in gray.

Figure 3.2

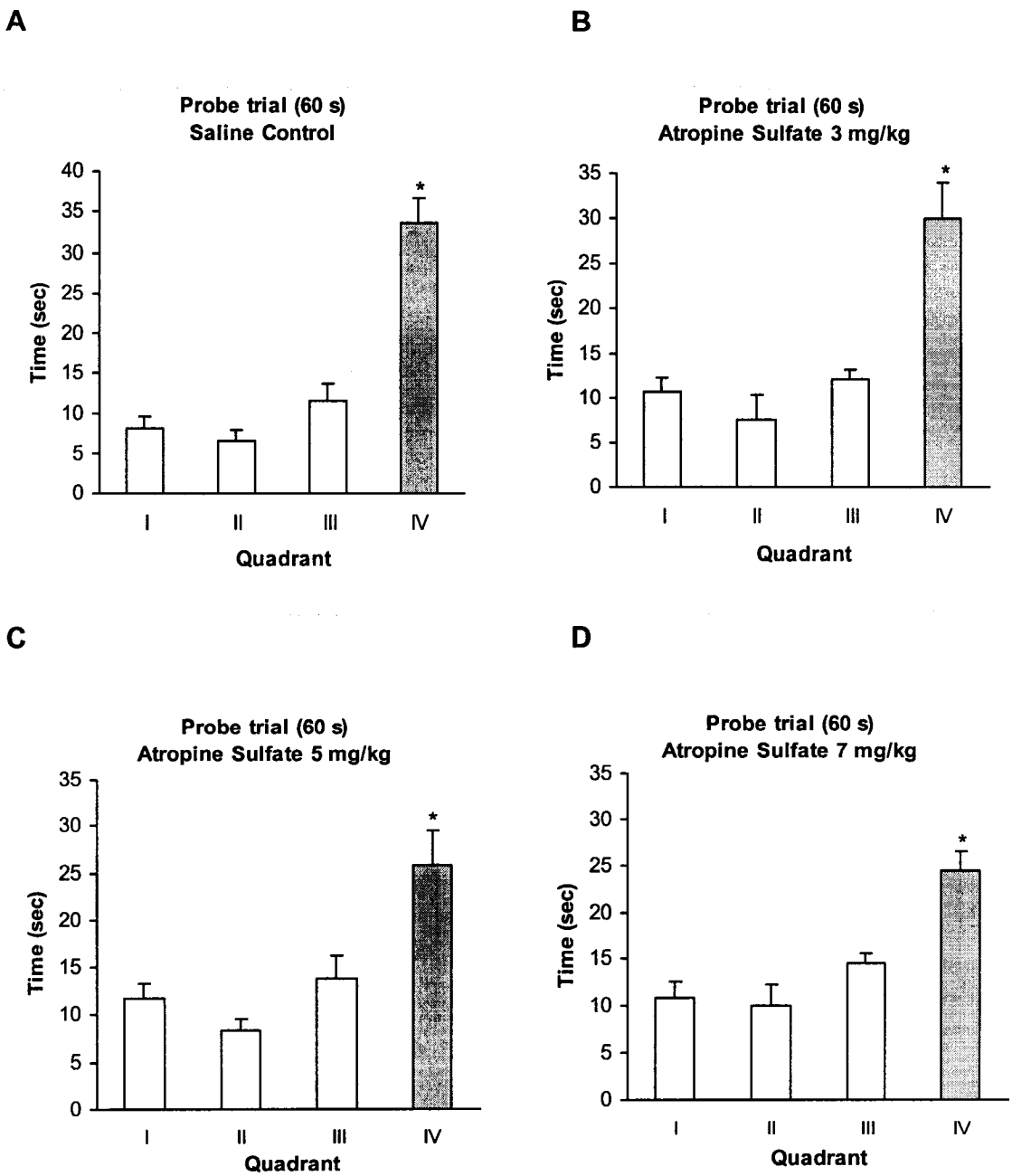

A
Probe trial (60 s)
Saline Control
Time (sec)
Quadrant
I
II
III
IV
B
Probe trial (60 s)
Atropine Sulfate 3 mg/kg
Time (sec)
Quadrant
C
Probe trial (60 s)
Atropine Sulfate 5 mg/kg
Time (sec)
Quadrant
D
Probe trial (60 s)
Atropine Sulfate 7 mg/kg
Time (sec)
Quadrant

Figure 3.3. Graphs show the average latencies per day for cued and spatial training of rats given distilled water or NPC 17742 (3, 4 or 5 mg/kg, $n = 8$ for each group). Panel A shows all groups. Panels B, C, and D show the control group compared with the 3, 4 and 5 mg/kg groups, respectivey. All groups were able to learn the cued task, with rats given 5 mg/kg being slower than controls. All groups also learned the spatial task. However there was an impairment in a dose-dependent manner with the 4 mg/kg group being slower than controls during the first four days of training, and with the 5 mg/kg being impaired troughout all training days.

Figure 3.3

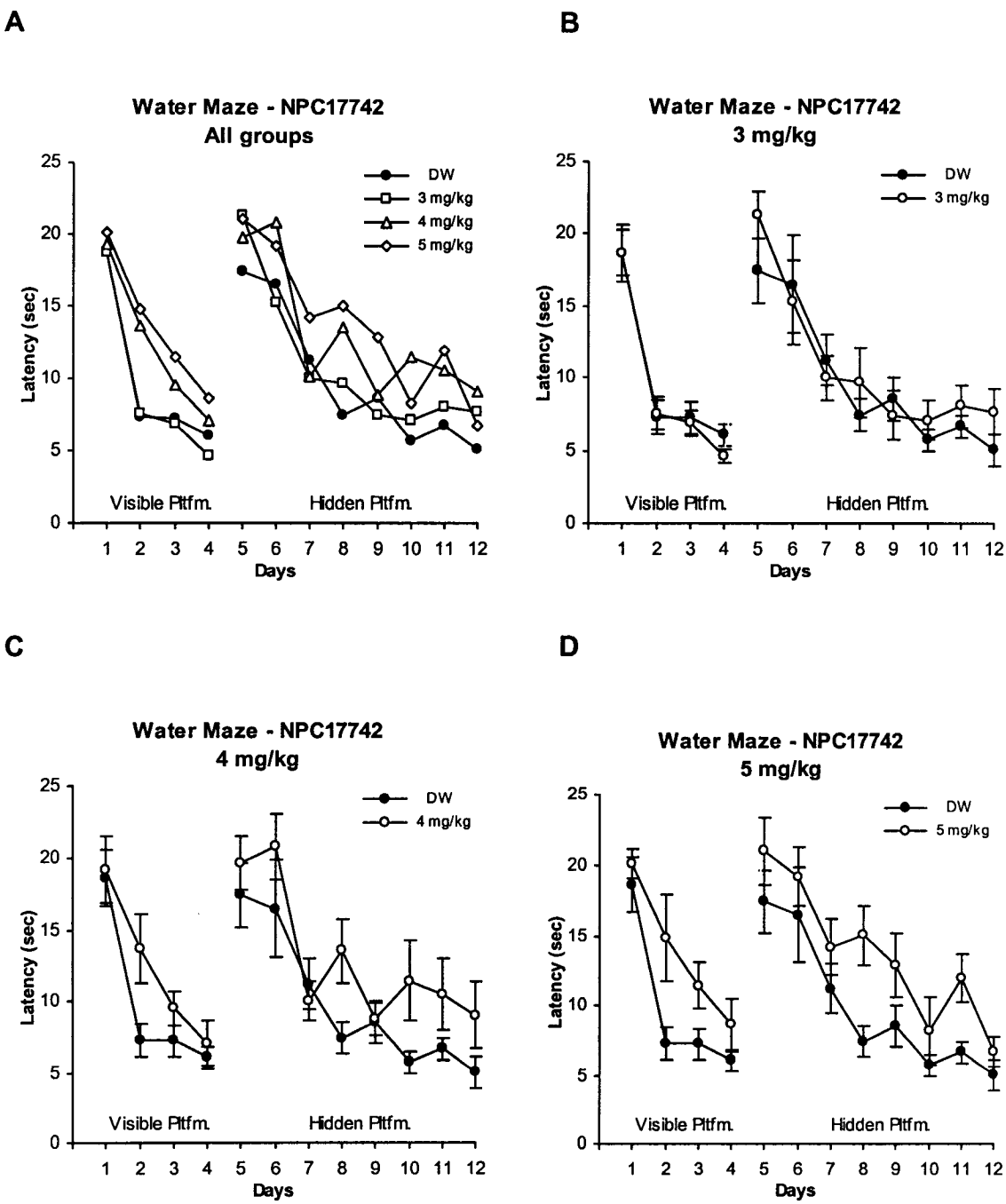

A
Water Maze - NPC17742
All groups
DW
3 mg/kg
4 mg/kg
5 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
B
Water Maze - NPC17742
3 mg/kg
DW
3 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
C
Water Maze - NPC17742
4 mg/kg
DW
4 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
D
Water Maze - NPC17742
5 mg/kg
DW
5 mg/kg
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days

Figure 3.4. Graphs show time spent in a quadrant for each group: distilled water (panel A), and 3 (panel B), 4 (panel C), or 5 mg/kg (panel D) of atropine sulfate. All groups spent more time on the target quadrant than in the others during the 60 s probe trial. Each bar shows a quadrant. The target quadrant is in gray.

Figure 3.4

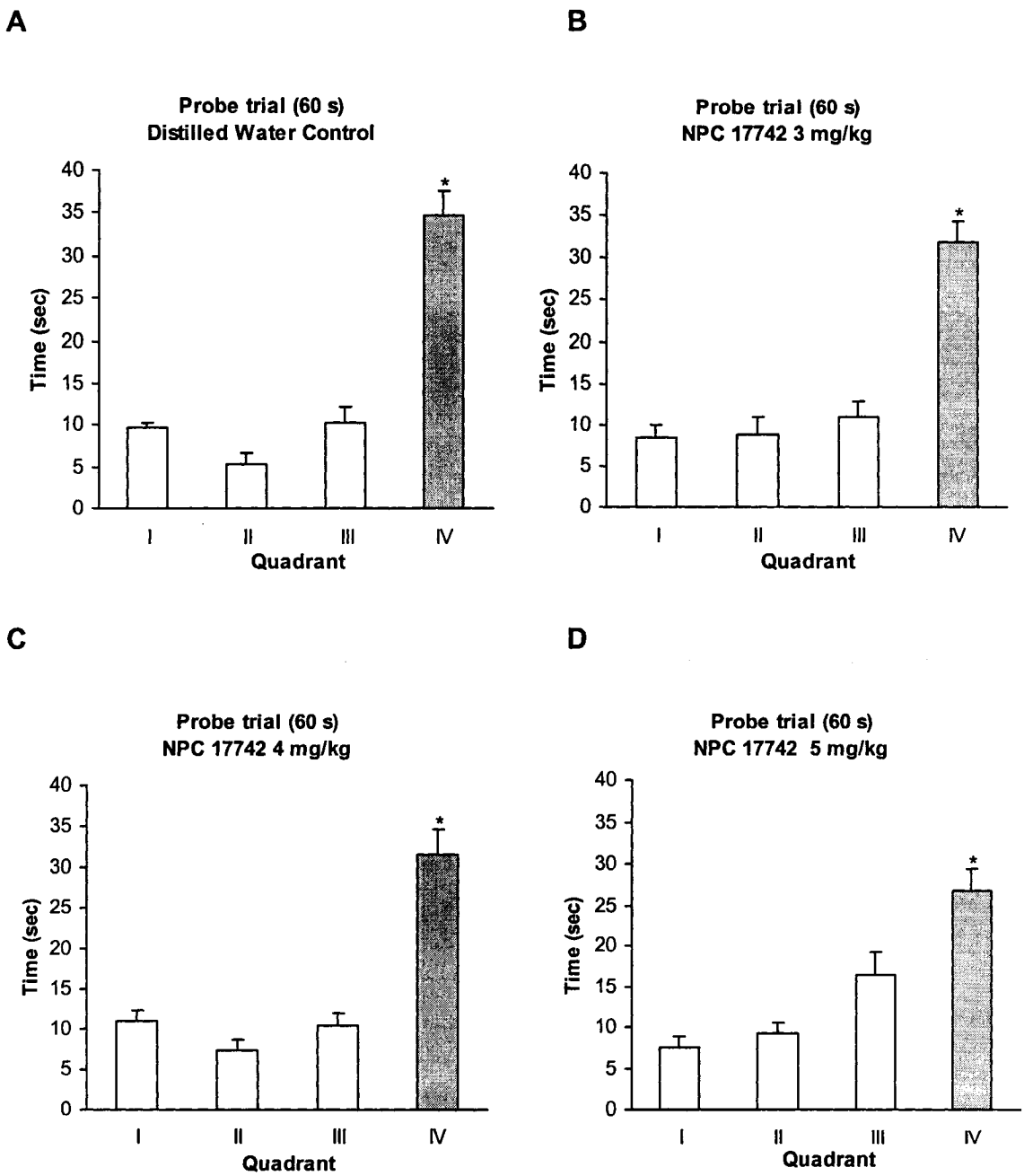

A
Probe trial (60 s)
Distilled Water Control
Time (sec)
Quadrant
I
II
III
IV
B
Probe trial (60 s)
NPC 17742 3 mg/kg
C
Probe trial (60 s)
NPC 17742 4 mg/kg
D
Probe trial (60 s)
NPC 17742 5 mg/kg

Figure 3.5. Graphs show the average latencies per day for cued and spatial training of rats given saline, atropine sulfate, NPC 17742, or a combination of atropine sulfate and NPC 17742 ($n = 8$ for each group). Panel A shows all groups. Panels B, C, and D show the saline group compared with the NPC 17742, atropine sulfate, and both drugs combined, respectivey. Rats given 3 mg/kg of NPC 17742 were impaired in the spatial, but not the cued task. Rats given atropine sulfate alone or in combination with NPC 17742 were not impaired in any of the tasks.

Figure 3.5

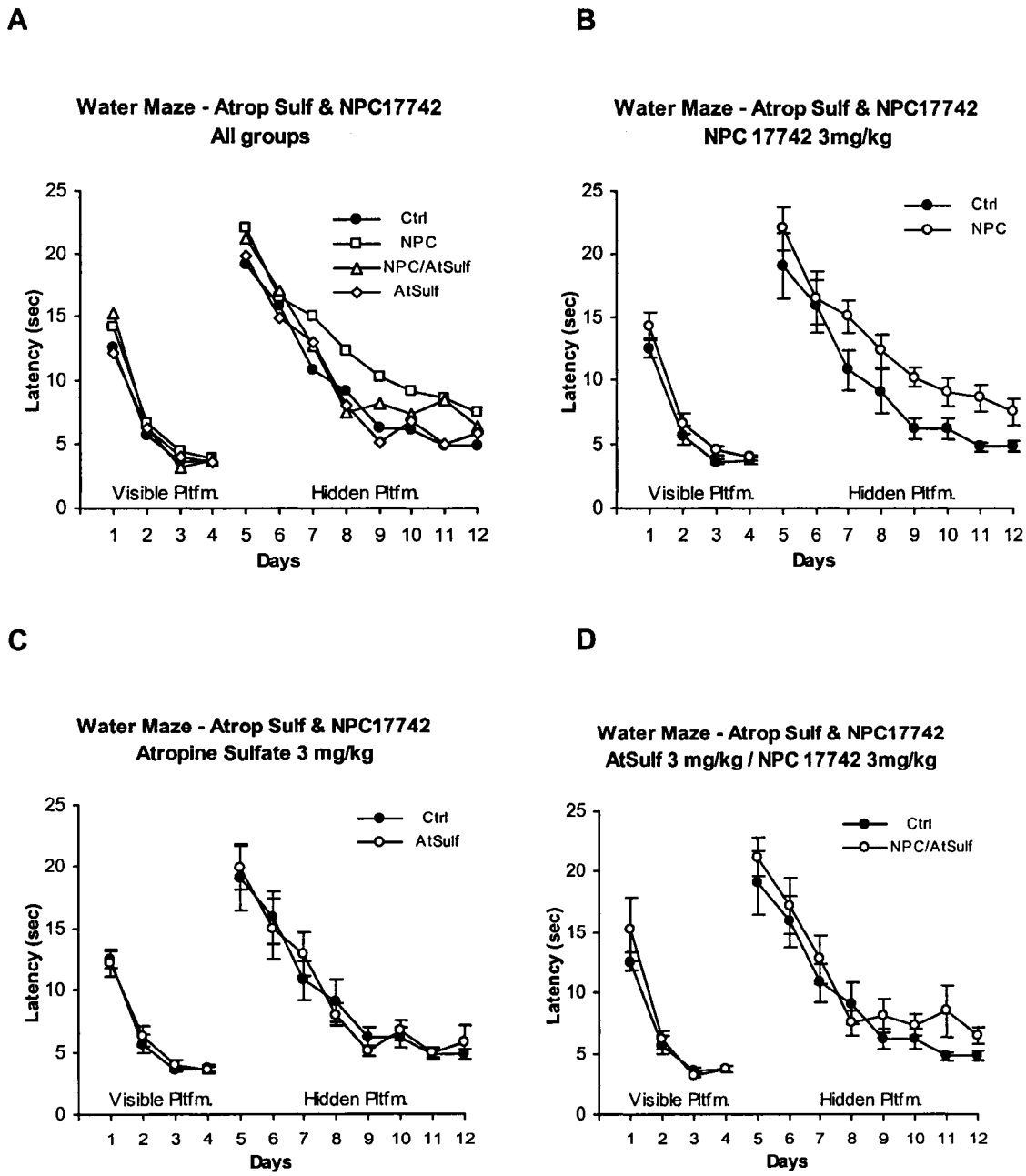

A
Water Maze - Atrop Sulf & NPC17742
All groups
Ctrl
NPC
NPC/AtSulf
AtSulf
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
B
Water Maze - Atrop Sulf & NPC17742
NPC 17742 3mg/kg
Ctrl
NPC
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
C
Water Maze - Atrop Sulf & NPC17742
Atropine Sulfate 3 mg/kg
Ctrl
AtSulf
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days
D
Water Maze - Atrop Sulf & NPC17742
AtSulf 3 mg/kg / NPC 17742 3mg/kg
Ctrl
NPC/AtSulf
Latency (sec)
Visible Pltfm.
Hidden Pltfm.
Days

Figure 3.6. Data for control and 3 mg/kg of NPC 17742 groups of Experiments 2 and 3 was combined. The graph show the combined average latencies per day for cued and spatial training of rats administered a vehicle or NPC 17742 (n = 16 for each group). Rats given 3 mg/kg of NPC 17742 were impaired in the spatial, but not the cued task.

Figure 3.6

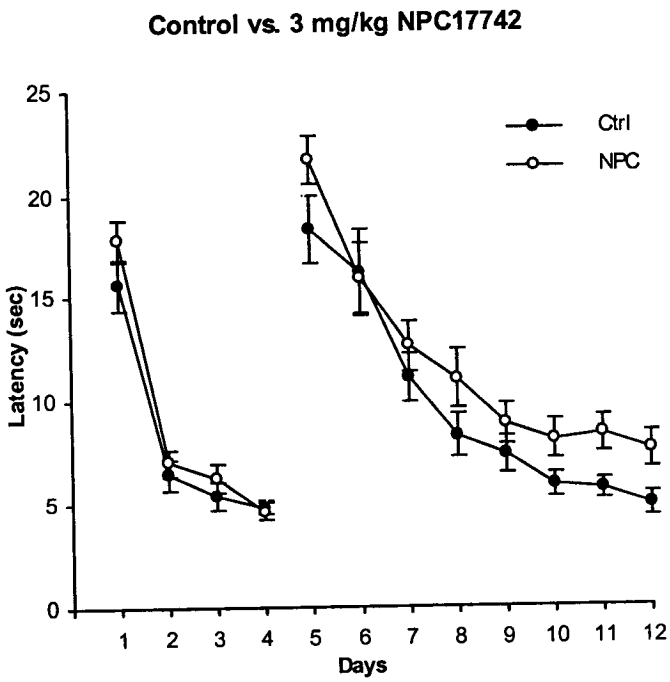
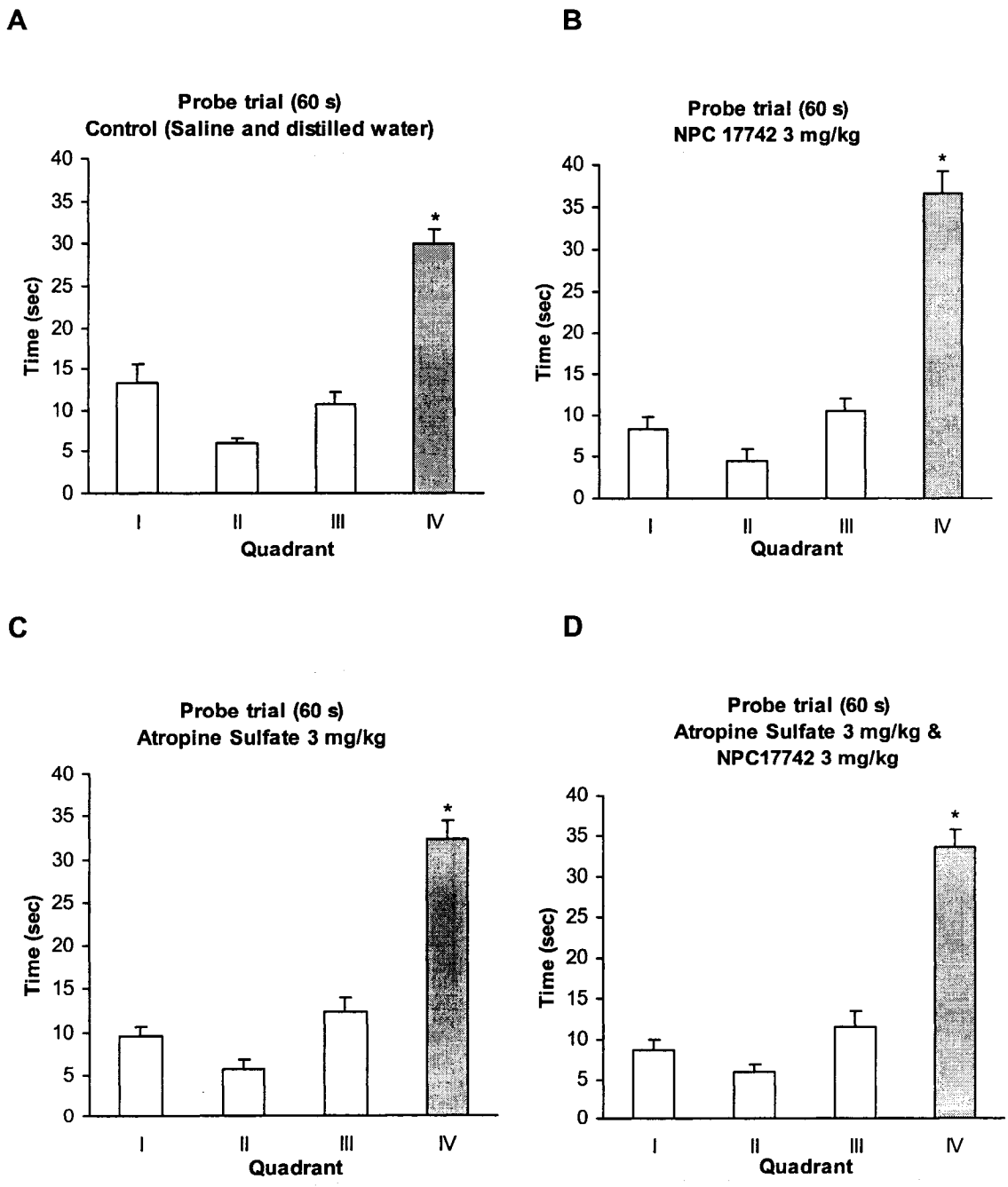

Control vs. 3 mg/kg NPC17742
Ctrl
NPC
Latency (sec)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
Days

Figure 3.7. Graphs show time spent in a quadrant for each group: control (panel A), NPC 17742 (panel B), atropine sulfate (panel C), and both antagonists combined (panel D). All groups spent more time on the target quadrant than in the others during the 60 s probe trial. Each bar shows a quadrant. The target quadrant is in gray.

Figure 3.7


A
Probe trial (60 s)
Control (Saline and distilled water)
Time (sec)
Quadrant
I
II
III
IV
B
Probe trial (60 s)
NPC 17742 3 mg/kg
C
Probe trial (60 s)
Atropine Sulfate 3 mg/kg
D
Probe trial (60 s)
Atropine Sulfate 3 mg/kg &
NPC17742 3 mg/kg

CHAPTER 4. A dose of competitive NMDA antagonist NPC 17742 that impairs primed burst potentiation and spatial learning in the water maze also impairs the acquisition but not performance on a working memory task in the radial arm maze

INTRODUCTION

In Chapter 2 it was shown that both the noncompetitive NMDA antagonist MK-801 and the competitive NMDA antagonist NPC 17742 impair the induction of PBP in the perforant path-dentate gyrus projection. These results correlate with specific learning impairments observed after systemic administration of the same drugs. MK-801 impairs the acquisition of spatial information in several behavioral tasks such as the water maze and the radial arm maze (Caramanos & Shapiro, 1994; Heale & Harley, 1990; Robinson, Crooks, Shinkman, & Gallagher, 1989; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). In Chapter 3 it was shown that the same dose of NPC 17742 that disrupts PBP (i.e., 5 mg/kg) also disrupts the learning of the location of a hidden but not a visible platform in the water maze. The effects of NPC 17742 on learning seem to be due to a disruption of NMDA receptor mediated synaptic transmission in the hippocampal system. Because of this and of the hypothesized role of the hippocampus in flexible learning, this study tested whether the same dose of NPC 17742 that impaired learning the location of a hidden platform would also disrupt learning of a spatial working memory task. Specifically, I tested the possibility that learning of spatial working memory task in the radial arm maze is disrupted by systemic injections of the antagonist. Pharmacological manipulations were done in naïve and pre-trained rats to explore possible behavioral dissociations.

Rodent spatial learning has been shown to be affected by treatments with an array of NMDA receptor antagonists using several behavioral paradigms. These paradigms are not

equivalent, and they measure distinct aspects of rodents' strategies for processing spatial information needed to solve a task (e.g., avoid an area inside a maze, search for food, escape from a platform). The radial arm maze is an extensively used task that evaluates the processing of spatial information on several levels (see Olton, Becker, & Handelmann, 1979). In this task, rats explore an elevated maze with equally spaced arms that project outward from a central platform to search for food located at the end of arms. Although the task has been used to assess cued and affective learning in addition to spatial learning (see McDonald & White, 1993), the focus here however will be on its use for the assessment of spatial information processing. When the task is used to measure spatial learning, hipothetically all baited arms have equal value. Intramaze cues are irrelevant for solving the task, navigation depending therefore on cues around the maze that remain in a constant location throughout training.

Although both the water maze and the radial maze measure spatial learning, the information provided by the use of these tasks is not redundant (see Hodges, 1996). The standard version of the water maze (Morris, 1981) allows to differentiate between learning about space and the procedures to solve the task by training animals to search for a hidden or visible platform. The task includes an aversive component (i.e., escape) and relies on the exploration of an open field. The standard version of the radial arm maze (Olton et al., 1979) allows to study working memory. The task requires that animals make multiple choices within the same trial. This imposes a level of complexity that other tasks requiring a single response during a trial, such as the water maze, do not have. In the radial arm maze, in addition to learning where food is located, an animal has to remember which arms were visited during the trial. Therefore, at least two types of processing are required to solve the

task efficiently: establishing a relationship between environmental stimuli to guide navigation (as in a cognitive map) and remembering which actions or strategies were implemented during a specific trial. The first type of information, known as reference memory, remains unchanged during training. The second type of information, known as working memory, can change from trial to trial, and therefore pertains to the current trial only.

There are two commonly used variations of the task: one where all arms are baited and another where only some of them are. When all arms are baited, the task, known as the 8/8 task when an eight-arm radial maze is used, measures spatial working memory. This type of processing uses intratrial specific information since an animal has to remember whether it visited an arm during a specific trial, ignoring visits made during previous trials. When only some of the arms are baited (known as the 4/8 task whenever only four arms are baited), the task tests for both reference and working memory. Reference memory entails the processing and storage of the location of food, which is always placed in the same four arms, across trials. Working memory entails remembering which of those four arms have been entered as a trial progresses.

The learning of a radial arm maze task is sensitive to manipulations of the hippocampal system. Lesions of this system produce deficits on spatial learning in the maze (Olton & Papas, 1979; McDonald & White, 1993). Pharmacological manipulations have also shown to disrupt learning. Infusions with NMDA antagonists MK-801, CPP, and APV produce deficits in the learning of a 4/8 task in unfamiliar environments (Caramanos & Shapiro, 1994; Shapiro & O'Connor, 1992; Ward, Mason, & Abraham, 1990). The combination of lesions and pharmacological interventions exacerbates the observed deficits.

Intraventricular treatment with MK-801 intensifies deficits in reference memory obtained after lesions of the entorhinal cortex (Zajaczkowski, Quack, & Danysz, 1996). Working memory is impaired in addition to reference memory in entorhinal cortex lesioned rats when MK-801 administration is systemic (Keseberg & Schmidt, 1995).

Some studies have shown that treatment with NMDA receptor antagonists can disrupt learning in tasks that involve working memory. For example, an array of NMDA antagonists that include CPP, MK-801, NPC 12626, and phencyclidine impair choice accuracy in a nonspatial delayed matching-to-sample working memory task (Pontecorvo, Clissold, White, & Ferkany, 1991). CPP and MK-801 also produce deficits on delayed conditional discrimination, another nonspatial task that depends upon working memory (Tan, Kirk, Abraham, & McNaughton, 1989). Similarly APV disrupts working memory in the radial arm maze, supporting the idea that NMDA receptors are also important for learning tasks requiring spatial working memory (Danysz, Wroblewski, & Costa, 1988). Finally, both MK-801 and CPP disrupt the 8/8 task on the radial arm maze (Ward, Mason, & Abraham, 1990).

When assessing the role of NMDA receptors in spatial working memory it might be difficult to conclude which component of processing is affected: spatial learning or working memory. A strategy that dissociates these processing components in the same task was implemented by Caramanos and Shapiro (1994). They showed that the NMDA antagonist MK-801 disrupts the reversal of spatial learning in the 4/8 radial arm maze task, but did not affect working memory: rats cannot learn new spatial information but can remember the place where food was located within a trial. They also found that rats trained on the same task were not impaired in a familiar environment after treatment with APV, but were unable to learn the same task in an unfamiliar environment. These results suggest that NMDA

receptors are important for learning but not for working memory once an environment is learned.

In this chapter the effects of the competitive antagonist NPC 17742 on spatial learning in rats trained in the 8/8 arm radial maze task was tested using the same dose that impaired PBP induction and spatial learning in the water maze (Chapters 2 and 3, respectively). First, rats were trained on the standard version of the 8/8 task after treatment with the antagonist to assess spatial learning. Then the effects of the antagonist on working memory were tested. For this, rats that were pretrained in a drug free condition in the standard 8/8 task until reaching a near errorless performance were treated with the antagonist and retested in the same environment in a variation of the task that includes a delay. Delays of various durations were introduced between the fourth and fifth choice within a trial to test whether and to what degree the NMDA receptor antagonist NPC 17742 affects the temporary storage of information within the same trial.

METHOD

Variations of the method used have been reported elsewhere (Caramanos & Shapiro, 1994; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992).

Subjects

Male Long-Evans rats (Charles River, St. Constant, Québec) were used. The rats weighed 275-300 g and were 3 months of age at the start of the experiment. The rats were housed individually in transparent cages, in a temperature-controlled room with a light cycle from 7 am to 7 pm. All animals had ad libitum access to food and water previous to the study.

Apparatus

An elevated radial arm maze was used to test spatial learning and memory. The maze consisted of an octagonal central platform (40 cm across) which was raised 60 cm from the floor. Eight equally spaced arms (55 cm long, 9 cm wide, with edges 2 cm high) projected from the platform. A food well (0.5 cm deep) was located 2 cm from the end of each arm. The maze was constructed of wood and painted black. Plexiglas walls surrounded the center platform. Eight Plexiglas gillotine doors controlled access to each arm. Each door was manipulated from a distance of several feet through plastic cables attached to the top of each door. Cues of various sorts (cardboard boxes, posters, bookshelfs, windows, and a radio, among others) were located around the testing room.

Drug treatment

Rats were assigned randomly to a control or a drug treatment group. Depending on the task used (see below), rats were treated during specific periods of the study. Rats were treated with either NPC 17742 (5 mg/kg, i.p.; $n = 8$) or a saline solution (i.p.; $n = 8$) 60 min before testing on the spatial tasks. During shaping all rats received a saline injection 60 min before testing.

Behavioral testing

Shaping. Rats were individually handled for 7 consecutive days, for 5 min every day. Starting on the first day of handling, rats were given daily limited amounts of food until they reached 85 % of their total free-feeding weight, which took 10-12 days. Afterwards, a controlled amount of food was given daily after testing, allowing each animal to gain 5 g per week. After reaching their target weight, rats were shaped for 6 days to enter each arm of the maze. On the first day of shaping, Froot Loops (Kellogs, Mississaugua, Ontario, Canada) pieces were placed in several areas of the maze, allowing two rats to explore the maze and

freely eat the food for 10 min. On the next 5 days rats were put individually in the center of the platform and allowed to explore the maze for 5 min with food located in the floor of the arms. While being shaped, rats were injected with saline 1 hr prior to maze exploration.

Standard 8/8 task. After the shaping phase rats were tested for spatial learning in the maze for 30 consecutive days. For a training trial the rat was put into the center of the platform for 10 s with all maze doors closed. Then all doors were opened simultaneously and the rat was allowed to search for eight Froot Loops' pieces hidden in the well at the end of each arm. A trial ended after a rat had made eight choices or 5 min had elapsed. A choice was counted after the rats' hindquarters passed the door to an arm. A working memory (WM) error was counted each time a rat entered an arm that had been visited previously during the same trial. Between rats the maze was cleaned for food particles.

8/8 task with delay. Rats were trained in the standard 8/8 task for 45 days until reaching near errorless performance (mean total WM errors per group was less than 0.1). On the last day of training rats were injected with saline to habituate them to the injection procedure. After this, rats were divided in two groups, each receiving either drug or saline, and tested in a variation of the task standard 8/8 task on which a delay was introduced between the fourth and fifth choices (see Olton & Markowska, 1993). For the delay rats were removed from both the maze and room and located into their home cage for the duration of the delay. Rats were tested for 3 days with a delay of 5 min. After this, rats were tested 3 more days with a delay of 30 min.

Statistical analyses

WM errors were counted for each rat for each trial and the mean number of errors was calculated for each day and for blocks of 5 or 3 consecutive days, for the standard 8/8

task or the 8/8 task with a delay, respectively. WM errors were counted in two ways: Total WM errors and WM errors made during the first eight arm entries. While the first measure assessed overall performance, the second measure was used as a means to examine impairments that occur at the beginning of a trial. A third measure, total choices made within a trial, assessed general exploratory behavior. The data were analyzed using repeated measures multivariate analysis of variance (ANOVA).

RESULTS

Standard 8/8 task

Rats injected with NPC 17742 (henceforth, NPC rats) were impaired in spatial learning compared to control rats (see Figures 4.1-4.3). Control rats made a mean of 0.5 WM errors per trial after 16 days of training, while NPC rats made on average two WM errors (see Figure 4.1). After 30 days (6 blocks) NPC rats were still significantly impaired: repeated measures multivariate ANOVA, effect of groups, $F(1, 14) = 14.75$, $p < .002$; interaction of groups and blocks, $F(5, 10) = 1.04$, $p = .44$ (see Figure 4.2, panel A). Analysis of the last block of trials shows that at the end of training NPC rats kept making more total WM errors than control rats: ANOVA for Block 6, $F(1, 14) = 9.59$, $p < .01$.

Analysis of WM errors made during the first eight choices showed that NPC rats made more WM errors than controls at the beginning of trials: repeated measures ANOVA, effect of groups, $F(1, 14) = 8.88$, $p < .002$; interaction of groups and blocks, $F(5, 10) = 1.1$, $p = .42$ (see Figure 4.2, panel B). Analysis of the last block of trials shows that at the end of training NPC rats kept making more WM errors than control rats during the first eight choices within a trial: ANOVA, effect of groups, $F(1, 14) = 9.52$, $p < .01$.

Analysis of the total entries made by rats showed that all animals explored the maze, visiting all arms at least once during a specific trial. All rats made fewer arm entries over time, with control rats having nearly errorless performance by the end of training with a mean of eight choices per trial (see Figure 4.3). During the experiment, NPC rats made more entries to arms than control rats: repeated measures ANOVA, effect of groups, $F(1, 14) = 12.2$, $p < .005$; interaction of groups and blocks, $F(5, 10) = 2.64$, $p = .09$. This difference between groups continued until the end of training. Analysis of Block 6 of trials shows that NPC rats continued making more entries until the end of the experiment: ANOVA, effect of groups, $F(1, 14) = 9.59$, $p < .01$.

8/8 task with delay

All rats were trained in the standard 8/8 task until reaching a near errorless performance of less than 0.1 WM errors per trial (see Figure 4.4). Previous to drug injection, groups did not differ in performance during the last three blocks of three days: repeated measures ANOVA for total WM errors, effect of groups, $F(1, 14) = 0.15$, $p = .7$. The introduction of a 5 min delay to the task did not alter rats' performance: ANOVA, Block 3 X Block of 5 min delay, $F(1, 15) = 0.84$, $p = .38$, for control rats; ANOVA, Block 3 X Block of 5 min delay, $F(1, 15) = 4.07$, $p = .06$, for NPC rats. The introduction of a 30 min delay did not alter rats' performance either: ANOVA, Block 3 X Block of 30 min delay, $F(1, 15) = 1$, $p = .33$, for control rats; ANOVA, Block 3 X Block of 30 min delay, $F(1, 15) = 3.56$, $p = .08$, for NPC rats. Groups did not differ at any of the two delays: for the 5 min delay, ANOVA, effect of groups, $F(1, 15) = 0.68$, $p = .43$; for the 30 min delay, ANOVA, effect of groups, $F(1, 15) = 1.3$, $p = .27$.

DISCUSSION

Systemic injections of a dose of NPC 17742 that impairs PBP, a short-term form of synaptic plasticity, also impairs the performance of a well-learned spatial working memory task in the radial arm maze. This impairment was assessed using several measures. The most standard measure commonly used in this paradigm, total WM errors, shows that NPC rats made more errors than control rats. However, the administration of NPC 17742 did not completely block learning since NPC rats were able to improve their performance across days. This implies that while NMDA receptors are necessary for learning the task, other mechanisms are at work in the processing and storage of spatial information that allow some degree of learning to occur. Alternatively, it can be argued that the block of the receptors was not complete, allowing for some residual learning.

Another measure used, the number of WM errors made during the first eight choices, allows a description of the impairment that can delineate some directions for further study. In an apparatus that allows for eight correct choices it would be expected that as more information accumulates during a trial (i.e., a growing list of visited arms), the degree of difficulty to decide which arm should be visited increases. Making more errors at the end of the trial could be indicative of a working memory deficit. If, on the other hand, animals persistently make more errors than controls at the beginning of the trial, then the impairment could be suggestive of a deficit in learning the set of spatial relationships that make up the environment and not necessarily of a disruption in keeping trial-specific information in working memory. If this were the case, the antagonist would be disrupting the ability to establish and/or remember a navigational map and not the ability of the animals to learn the working memory strategy. Since in this study NPC rats consistently made more WM errors

during the first eight choices than control rats, it could be possible that treated rats were impaired in their ability to build a spatial representation of the environment in the same manner as in Chapter 3 NPC rats were impaired in the ability to learn the location of a hidden platform in the water maze. However, it was necessary to test this suggestion further to discern whether the observed defict was one of working memory or one of the construction of a spatial representation. Because of this the second behavioral manipulation was introduced.

A question that might remain about the deficit observed in the first study is whether the results could be attributed to other factors such as locomotor side effects. This is so because some NMDA antagonists have been associated with akinesia, hyperactivity or changes in locomotion (Hargreaves & Cain, 1992, 1995; Whishaw & Auer, 1989). The results presented here as well as those in Chapter 3 present that as a remote possibility. In Chapter 3 rats injected with the same dose of the antagonist were able to quickly learn a cued task. Still, since swimming and climbing onto one visually salient platform, and walking to and inside an arm do not require the same strategies and are based on different incentives (escaping the water vs. finding food) the question still would need further analysis. The third measure used to assess performance in the first study, the total amount of arm entries during a trial, served as a measure of general activity that helps to address the matter of a locomotor impairment. The author previously observed in the same task that rats injected with competitive NMDA receptor antagonist CGP 39551 in a dose that blocks long-term potentiation in the dentate gyrus (Maren, Baudry, & Thompson, 1992) became ataxic and were barely able to efficiently explore the radial maze (e.g., became sluggish, were not able to walk in a straight pattern, stopped continuously, fell on their sides or from the maze arms,

and clashed persistently against the Plexiglas wall that surrounds the center platform; data not shown). Because of this general activity and arm entries were examined. NPC rats did not present any noticeable locomotor changes, nor did they make *fewer* entries to arms than control rats. On the contrary, NPC rats explored the maze visiting all arms with roughly the same or higher frequency than controls. NPC 17742 therefore does not seem to impair locomotion or general exploratory behavior in the radial arm maze, but choice accuracy.

In general, the results from the first study point to a deficit of a declarative nature. However, the results still leave unanswered the question whether NMDA receptors are important for the learning of spatial information or for processing the rapidly changing contingencies associated with working memory. From this experiment alone it might be infered, although not answered conclusively that NPC 17742 impaired learning about the spatial attributes of the environment and not necessarily working memory. Therefore it was necessary to devise a similar learning task that could allow to dissociate these two learning components.

The second study, the testing of drug treated rats in a familiar environment with a delay allowed to assess the functional distinction between learning of a spatial representation and the processing of trial-specific spatial information. For this study, after rats reached a near errorless performance, a drug treatment and a delay were introduced. The delay added another level of difficulty to the 8/8 task. The task places a higher level of difficulty than the 4/8 task in terms of the amount of information that has to be remembered within a trial (i.e., more arms with food). This variation has been shown to be sensitive to hippocampal dysfunction (Olton & Markowska, 1993). The delay was added for increasing the difficulty in a qualitatively different dimension, time. However, neither a longer list of arms nor a 5

min delay between the fourth and fifth choices mattered since rats treated with the antagonist were not affected. A substantially longer delay (30 min) did not affect performance of either control or NPC rats. The results of this second study therefore suggest that NMDA receptors are not necessary for the temporary processing of specific events once a spatial representation is learned.

The results of this study correlate with LTP saturation studies where after repeated high frequency stimulation of perforant path-dentate gyrus cells, rats cannot learn new spatial information but can perform a spatial working memory task on a radial-arm maze (McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986). LTP depends on NMDA transmission (Harris, Ganong, & Cotman, 1984; Morris, Anderson, Lynch, & Baudry, 1986) and is associated with increases in glutamate release (Bliss, 1990; Bliss, Errington, Laroche, & Lynch, 1987; Bliss, Errington, & Lynch, 1990; Malgaroli et al., 1995; Malinow & Tsien, 1990). It seems that the NMDA receptor-mediated synaptic changes that occur in the hippocampal system are necessary for spatial learning, but not for spatial working memory that operates on already known spatial information. Overall, the results show that NMDA receptors are necessary for learning the spatial representations upon which the working memory system operates, but once space is learned the working memory system is not NMDA dependent.

These results are consistent with previous findings (Shapiro & O'Connor, 1992) showing that the noncompetitive NMDA antagonist MK-801 affects spatial working memory in a novel but not in a familiar environment. The combined electrophysiological and behavioral data (Chapters 2-4) are consistent with studies and suggestions that NMDA receptors in the hippocampal system seem to be necessary for the learning of spatial

representations or at least episodic information (Morris, Davis, & Butcher, 1990; Richter-Levin, Canevari, & Bliss, 1995; Shapiro & Eichenbaum, 1999; Teyler, 1987; Teyler & DiScenna, 1987).

These inferences are related to the role of NMDA receptors and cannot generalize to the whole hippocampal system. For example, the results contrast with studies where after extensive training in the radial maze with only some arms baited, rats which undergo fimbria-fornix lesions suffer working memory deficits (Olton & Papas, 1979). Therefore, while the hippocampus seems to be necessary for performance in a working memory task, NMDA receptors seem to be necessary only for the acquisition of the task.

In general, the results suggest that NMDA receptors are important for the learning of complex multi-stimuli spatial representations that might involve frequently changing conditions. Thus, the receptors would be relevant for relating environmental stimuli in the form of a representation (reference memory) where relevant navigational-related aspects are stable. Other neural mechanisms would take into account the experience-dependent changes that occur in a new environment that is being learned, allowing the animal to efficently accomplish a set of goals in an economic way (e.g., searching for food only once in different locations). In this sense the learning of a spatial representation does not involve a rigid set of relationships among stimuli, but a more flexible one where navigation involves discriminating between essential elements that would define a map, and constantly changing situations related to specific aspects of the map.

But, what aspects of the environment are relevant for creating a stable representation? The next chapter deals with this question. By changing environmental conditions in multiple ways I explored what is relevant for rats in the learning of a spatial working memory task.

The role of NMDA receptors in the processing of specific spatial attributes of an environment was also explored.

Figure 4.1. Rats injected with NPC 17742 ($n = 8$) made more WM errors per day than rats injected with saline ($n = 8$). Each circle represents the mean errors per day. After 18 days of training saline rats acquired the task, making an average of 0.4 WM errors. Although NPC rats improved, they never reached that level of performance.

Figure 4.1

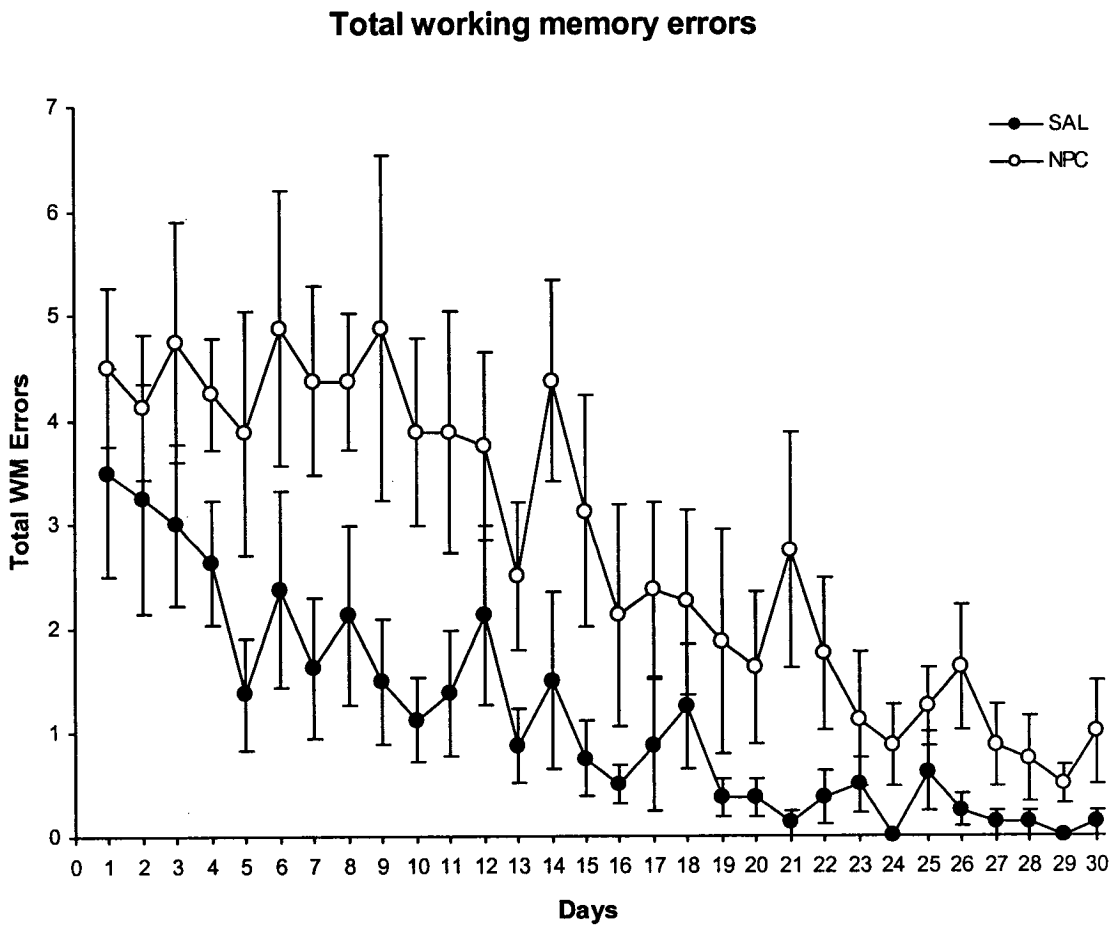

Total working memory errors
SAL
NPC
Total WM Errors
0
1
2
3
4
5
6
7
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Days

Figure 4.2. Rats injected with NPC 17742 made more WM errors during the trial (panel A) and during the first eight choices (panel B) than rats injected with saline. Each circle represents the mean errors per block of 5 days of training. Although all rats improved their performance, with control rats reaching a near errorless performance by Block 6, NPC rats consistently made more errors until the end of training.

Figure 4.2

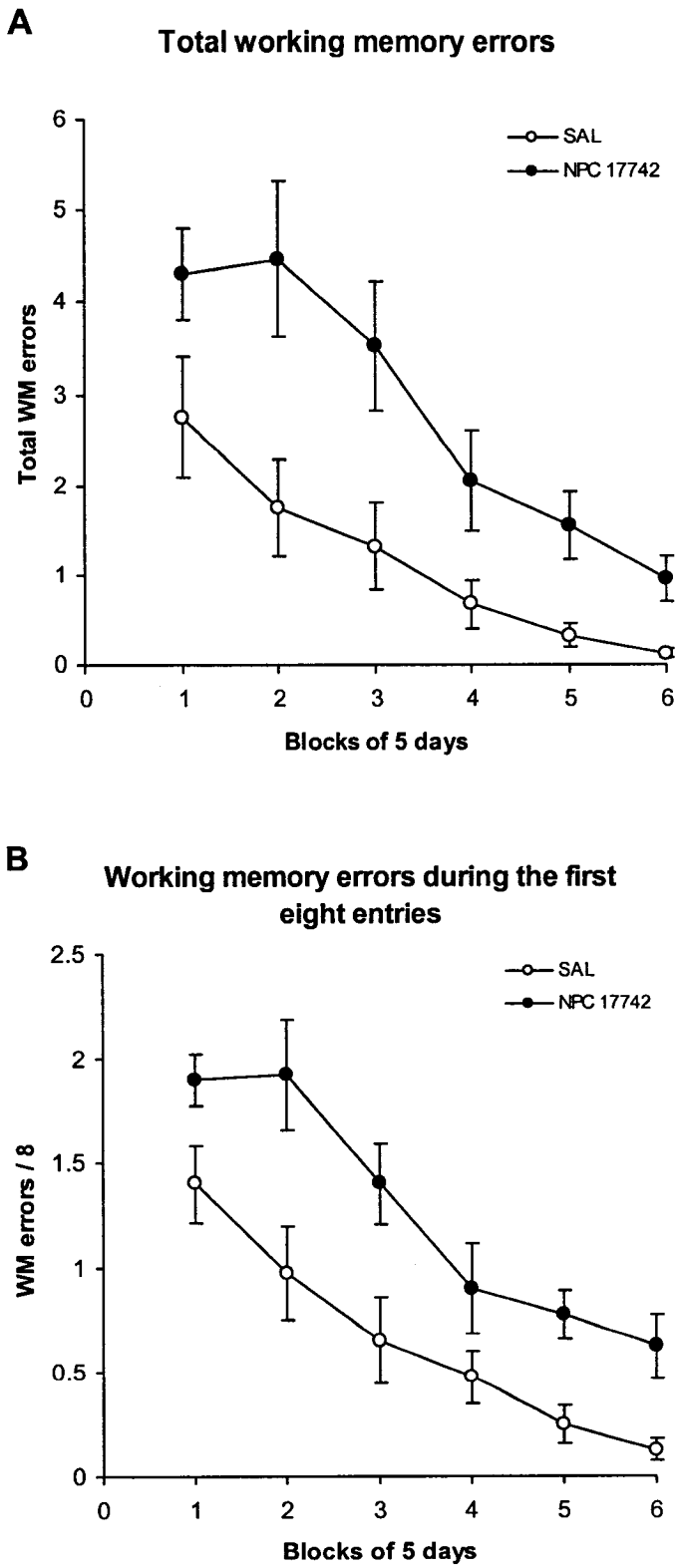

A
Total working memory errors
SAL
NPC 17742
Total WM errors
Blocks of 5 days
B
Working memory errors during the first eight entries
SAL
NPC 17742
WM errors / 8
Blocks of 5 days

Figure 4.3. Rats injected with NPC 17742 made more entries to the arms than rats injected with saline. Each circle represents the mean number of entries per block of 5 days of training. Saline rats made one entry by Block 2, while NPC rats reached the same level on Block 5.

Figure 4.3

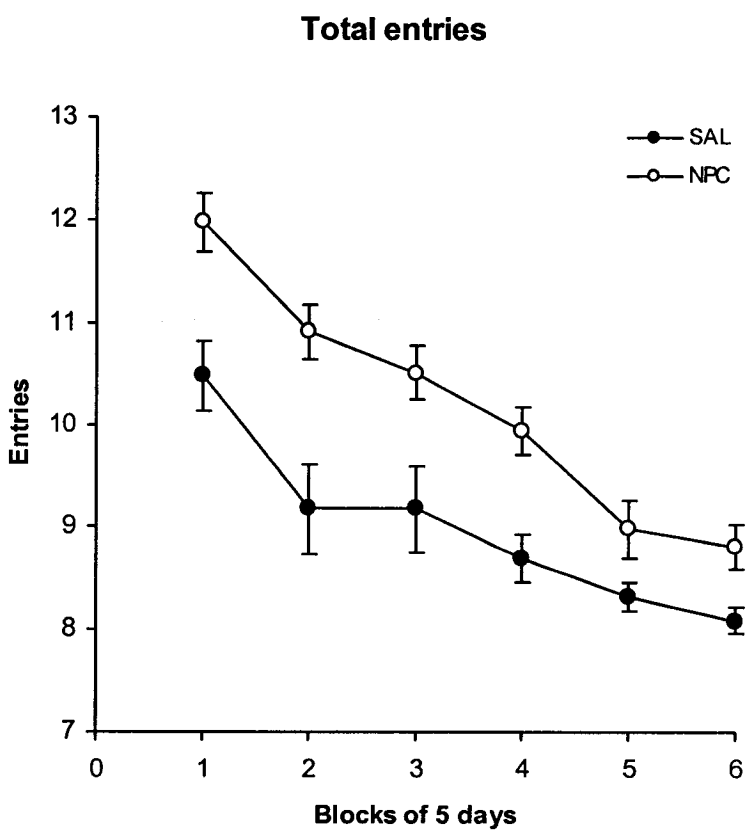
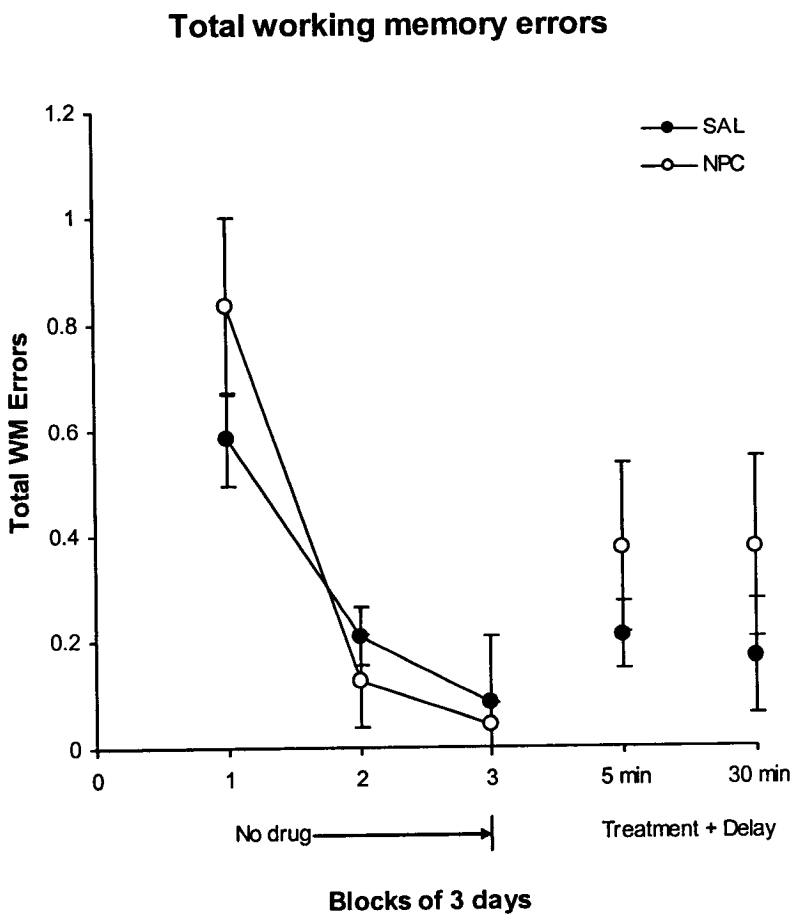

Total entries
SAL
NPC
Entries
13
12
11
10
9
8
7
0
1
2
3
4
5
6
Blocks of 5 days

Figure 4.4. Effects of delays of 5 or 30 min between the fourth and fifth choice in the performance of extensively trained rats after injections of NPC 17742. Each circle represents the mean working memory errors made per trial on a block of 3 days. The first three blocks show days 37-45 of a drug free performance of rats trained in the standard version of the 8/8 task in the radial arm maze task. The fourth and fifth blocks show the effects of a 5 or 30 min delay, respectively, in rats treated with either drug ($n = 8$) or vehichle ($n = 8$). The delay did not affect performance substantially. Groups did not differ neither in the drug free condition nor in any of the two delays.

Figure 4.4


Total working memory errors
SAL
NPC
Total WM Errors
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
5 min
30 min
No drug
Treatment + Delay
Blocks of 3 days

CHAPTER 5. Distal cues, but not their spatial organization or room geometry, are crucial for working memory performance in the radial maze

INTRODUCTION

Spatial learning, as assessed by a standard 8/8 task in the radial maze in Chapter 4, is impaired in rats given a dose of competitive NMDA receptor antagonist NPC 17742 that impairs PBP and spatial learning in the water maze. Administration of the antagonist in well-trained rats did not affect performance even when a 30 min delay was imposed between the fourth and fifth choices. The results suggest that blocking of NMDA receptors impairs learning the characteristics of the environment upon which spatial working memory operates. Once an environment is known, NMDA receptors do not seem to be relevant for spatial working memory. This is consistent with a study by Shapiro and O'Connor (1992) in which the administration of noncompetitive NMDA receptor antagonist MK-801 causes impairments in the 8/8 task in animals tested in a novel environment. This correlates with electrophysiological studies in which doses of MK-801 that impair spatial learning also attenuate PBP (see Chapter 2; Hargreaves, Côté, & Shapiro, 1997), and place field stabilization (Austin, Fortin, & Shapiro, 1990). The impairments observed with this drug do not seem to be of procedural learning, because pre-trained rats given NMDA antagonists perform the standard 8/8 radial arm maze task normally in a familiar room, but fail to learn the same task in an unfamiliar room (Shapiro & O'Connor, 1992). Because the same procedures and movements are required to solve the task in both rooms, learning about the surrounding stimuli in a new room must be impaired by the drug.

Most studies on hippocampal-dependent learning (for a review of those, see Chapter 1), as well as the studies presented in the previous chapters, assess electrophysiological and

neuropharmacological correlates of the learning of tasks that require the processing and storage of mostly static spatial information. This is because the primary goal of these studies is to understand some of the brain mechanisms involved in the representation of one environment over time. However, these studies do not seek to understand which aspects of that environment are more relevant during learning, nor they assess the adaptations that an organism undergoes whenever the spatial attributes of an environment change. Some studies however have delved into this strategy. For example, changes in the environment after learning a task have been in the form of introducing rats to an unfamiliar environment to study the role of either the hippocampus or NMDA receptors (Caramanos & Shapiro, 1994; Shapiro & O'Connor, 1992). Some hippocampal single-unit recordings have employed the technique of making changes in the environment once place fields have stabilized (Hetherington & Shapiro, 1997; Muller & Kubie, 1987; O'Keefe & Conway, 1978) or during learning (O'Keefe & Speakman, 1987). Some of these changes are in the form of cue subtraction, rotation or substitution.

The goal of the present experiments was to learn which stimuli were important for well-trained rats to perform WM tasks in the eight arm radial maze. The study of rodent spatial navigation assumes the existence of the formation of a cognitive map, or a plan where navigation depends on various environmental cues and the relationship between them (Tolman, 1948; Nadel, 1991; O'Keefe & Nadel, 1978). Single unit recording studies have shed some light into what aspects of space animals seem to process. By performing multiple manipulations on the environment we tested what elements animals use once they have been extensively trained. The extensive training and near errorless performance assumes the existence of a well defined spatial representation in these rats.

METHOD

The method described here is a variation of the standard procedure used in Chapter 4. Variations of this method have been reported elsewhere (Caramanos & Shapiro, 1994; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992).

Subjects

Male Long-Evans rats (Charles Rivers, St. Constant, Québec) were used. The rats weighed 275-300 g and were three months of age at the start of the experiment. The rats were housed individually in transparent cages, in a temperature-controlled room with a light cycle from 7 am to 7 pm. All animals had ad libitum access to food and water previous to the study.

Apparatus

Two elevated eight-arm radial mazes located in different rooms (a total of three) were used to test spatial learning and memory. Each maze consisted of an octagonal central platform (40 cm across) which was raised 60 cm from the floor. Eight equally spaced arms (55 cm long, 9 cm wide, with edges 2 cm high) projected from the platform. A food well (0.5 cm deep) was located 2 cm from the end of each arm. The mazes were constructed with sanded wood and painted either black or gray. Plexiglas walls surrounded the platform. Eight Plexiglas gillotine doors controlled access to each arm. Each door was manipulated from a distance of several feet through plastic cables attached to the top of each door.

To identify the factors required for good WM performance in well-trained rats, one radial maze was placed in a cue-controlled environment designed to allow the same manipulations that disrupt hippocampal place field activity: changes to room geometry and stimuli configuration. The maze was surrounded by black curtains that hung from two sets

of runners to change the shape of the room from square to circular. The curtains initially defined a square room, with each "wall" holding a prominent and visually distinct distal cue (a white pillowcase, a shopping bag, a tridimensional aluminum foil covered object, and a poster).

The second maze was located at different times during the study in two other rooms with fixed cues of various sorts (cardboard boxes, posters, bookshelfs, windows, and a radio, among others) located around the room.

Drug treatment

At several points during the course of the experiments rats were submitted to injections of a drug or vehicle. Rats were assigned randomly to a control ($n = 8$) or a drug treatment group ($n = 8$). The drug used was non-competitive NMDA antagonist MK-801 (0.80 mg/kg, i.p.). Treatments were given 60 min before testing on the spatial task. During each shaping session all rats received a saline injection 60 min before testing.

to resemble those that influence hippocampal place fields (see Shapiro, Tanila, & Eichenbaum,1997, for variations of this procedure). Entries into previously visited arms during the first eight choices were counted as working memory (WM) errors.

Two sets of probe tests were done (see Table 5.1 for a summary of tests). The first set of probe tests altered the testing room *before* the rats were exposed to that environment. The second set of probe tests altered the testing room *after* the rats were exposed to the maze and allowed to chose four arms.

The first set of probe tests had the purpose of assessing the effects of environmental manipulations on recently acquired strategies for spatial navigation. A description of each manipulation follows.

The first probe test was done to evaluate the original hypothesis by Tolman (1948) that rats organize cues in the environment as a cognitive map where cues are processed in terms of distance, order, and location in relation to each other. For this purpose the distal cues were scrambled to create a new configuration.

The second probe test assessed the possibility that room geometry might be relevant in well-trained rats for spatial navigation. For this purpose the original cue configuration was kept, but the curtains formed a circular room.

The third probe test introduced a new level of complexity to the environment. For this the configuration of cues used in the first probe test was put into the circular room, therefore changing all salient visual characteristics of the environment.

The fourth probe test investigated the need of visual cues in well-trained rats. For this purpose all distal cues were removed from the "walls".

The fifth probe test evaluated the hypothesis that rats in an unfamiliar environment have to learn new information in terms of visual cues and the organization among them. For this a new cue, a white paper stripe in one of the walls, replaced all previously used cues.

The sixth probe test was done with a similar purpose as the fifth probe test: assess rats' performance in an unfamiliar environment. For this probe test however all conditions changed since rats were tested in an unfamiliar room.

The second set of probe tests was done with the purpose of assessing spatial working memory in well-trained rats. For these tests the testing room was altered (i.e., cues were scrambled) *after* the rats were exposed to the maze and allowed to choose four arms. Therefore, rats would have to solve the task using conflicting information *within*, but not between trials. Before getting retested with a second set of probe tests rats were given 8 days of additional training in the original conditions that occurred before testing in the first set of probe trials to ensure asymptotic levels of performance.

The first probe test of the second set was done with the purpose of evaluating spatial working memory that includes changes in the environment during a single trial. For this the rats were given four choices and then were removed from the maze for 10 min. During this delay the distal cue configuration was altered. The rats were returned to the maze after the delay and allowed to complete the task.

The second probe test of the second set was done with a similar purpose as the first probe test. However, for this test the rats were given four choices, were removed from the maze for 1 hr, and then returned to the maze.

The third probe test of the second set was done with the purpose of evaluating spatial working memory with a delay between choices combined with cue rotations. The rats were given four choices and then were removed from the maze for 1 hr. During this time the constellation of distal stimuli was rotated 90 degrees counterclockwise. Each rat was returned to the maze from an entrance 90 degrees counterclockwise from the standard entrance to the room.

Drug tests

After 6 months of training, the rats were randomly assigned to either a saline or an MK-801 (0.08 mg/kg, i.p.) treatment subgroup. The identical drug treatment given to the MK-801 group blocked the induction of PBP in behaving rats of the same strain and sex (see Chapter 2). The rats were tested in both the familiar room with the curtains and then yet another unfamiliar environment (a third room not used for either training or any of the probe tests mentioned before).

Statistical analyses

Statistical tests were performed using Friedman's analysis of variance (ANOVA) for comparisons of probe tests in a within-subjects design. Measures were WM errors and consecutive visits to adjacent arms Multivariate ANOVAs were performed for comparisons between groups in the tests involving a drug treatment.

RESULTS

Training in the standard 8/8 task with no drug treatment

After 60 days of training rats reached a near errorless performance. On average they made less than 0.1 errors during the last 3 days of testing. This was used as the standard to compare performance in the six tests of the first set of probe tests (see Figure 5.1).

Probe tests

First set of probe tests

On the first probe test, the interchange of cues did not affect rats performance: Friedmann test, $p = .32$ (see Figure 5.1). Results were similar in the second probe test. Change in room geometry did not affect performance: Friedmann test, $p = .32$. The combination of both cue interchange and room geometry in the third probe test still did not have any effect on performance: Friedmann test, $p = .32$.

The removal of cues and their substitution with a new set in the fourth probe test caused animals to make more WM errors: Friedmann test, $p < .01$. However, when the new cues were removed and the original ones were still left out of the room, performance returned to its' original state: Friedmann test, $p = .32$.

In the sixth probe trial of the set, where rats were tested in the same task in a new room, performance was affected with rats making more WM errors than in the original training in the first room: Friedmann test, $p < .001$.

Second set of probe tests

On the first set of probe tests the cue interchange after the fourth choice, with a 10 min delay, did not affect performance: Friedmann test, $p = .99$ (see Figure 5.2). Increasing the delay to 1 hr in the second probe test did not have an effect in performance either: Friedmann test, $p = .32$. However, when the procedure of the second probe test was changed

in the next one by rotating cues and entrance to the testing room by 90 degrees 1 hr after four choices, rats made more WM errors than in the original training conditions given right before the second set of probe trials: Friedmann test, $p < .01$.

Assessment of consecutive visits to adjacent arms

To evaluate whether rats employed a stereotypical foraging strategy, the number of consecutive visits made to an adjacent arm was computed for the two sets of probe trials and compared to performance in the original training room (see Figure 5.3). Friedmann tests showed that rats did not change of strategy for any of the probe tests.

Drug tests

Treatment with MK-801 impaired performance only in a new room. When tested in the familiar environment, the performance of MK-801 treated rats did not differ from that of controls: ANOVA, $F(1, 13) = 0.02$, $p = .89$ (see Figure 5.4). When tested in a new environment though MK-801 treated rats were impaired compared to controls: ANOVA, $F(1, 13) = 6.1$, $p < .05$. Rats given saline performed well almost immediately in the new environment, demonstrating a learning set for the standard 8/8 radial arm maze task after repeated testing in unfamiliar rooms: ANOVA for saline rats only, $F(1, 6) = 0.013$, $p = .913$.

DISCUSSION

In the first set of probe trials the strategies that well-trained rats might use to solve a spatial task were assessed by systematically changing the spatial attributes that are commonly assumed to be used by rats during spatial navigation. Since changes in the environment failed to increase errors significatively, the results show that once a spatial representation is learned rats performance is very resilient to most environmental manipulations. This could mean that the representation of the environment is very complex,

with many other smaller elements in the environment being sufficient for the activation of the representation during navigation. First, neither the change in the position of stimuli in relationship to one another, nor the elimination of all salient cues affected navigation. Changes in room geometry did not affect performance either. The combination of some of these manipulations (i.e., changes in room geometry *with* the interchange of cues) was not effective either in affecting rats' navigation. However, the introduction of new information in the form of unfamiliar cues had a significant effect. First, the elimination of familiar stimuli and their substitution with new ones had the effect of increasing WM errors. Second, the testing of rats in an unfamiliar (i.e., new) environment affected performance in a similar manner.

In general, the first set of probe trials show that as long as new information in the form of cues is not added, rats can perform a spatial working memory task relatively well. Neither subtraction nor changes in information seem to have a noticeable effect on performance. It seems that after extensive training navigation depends on small subsets of the environment that are sufficient for the animal to locate a reward in the correct location of an ambiguous environment. There is converging evidence with single unit recording studies that show that place fields remain stable after removal of cues in a controlled environment (Muller & Kubie, 1987; O'Keefe & Conway, 1978). This is also consistent with studies that show that the firing patterns of some hippocampal cells that are related to particular locations of an environment, such as the background, remain virtually unchanged after manipulations to that environment (O'Keefe & Speakman, 1987).

The second set of probe trials introduced another level of complexity to the environmental manipulations by imposing a delay between the fourth and fifth choices. The

introduction of a delay in a radial arm maze task has been shown to affect performance in animals with hippocampal lesions (Olton & Markowska, 1993). Here though, the addition of a temporal component did not affect performance. Similar to the results in Chapter 4 with well-trained rats, the introduction of a 30 min delay in the standard 8/8 task did not affect performance of rats regardless of treatment (NPC 17742 or saline). For this study the delay was increased up to 1 hr and cues were interchanged of position. As in the radial maze study in NPC 17742 treated rats, MK-801 had no major consequence in performance, which shows that well-trained rats can easily keep a record of their experiences in a well-known environment for long periods of time, even when cues are interchanged of position. As in the first set of probe trials, small sets of information might be sufficient for navigation.

The results of the last probe trial of the second set suggest that the animals' position in relationship to the environment is a key element for successful navigation even in well known environments. When entrance to the room *and* the configuration of cues is rotated after the first four choices, performance is affected. In this case, the results contrast with the first set of probe trials were cues were scrambled without any noticeable effect. When starting position changes regarding the environment, a new type of information is introduced that affects the navigational strategy. This is consistent with single unit recording studies that show that place fields occur in a particular location only when rats are facing a specific cue (O'Keefe & Dostrovsky, 1971). The increase in errors after rats were started from a different location is also consistent with studies in which the firing of place cells changed not necessarily after cues were rotated inside a recording chamber or new cues were added, but after rats were started from a new location (Sharp, Kubie, & Muller, 1990). Therefore, spatial navigation in well-trained rats seems to depend on starting location with respect to

presumably at least one familiar cue (regardless of whether it is salient or from the background).

Rats do not seem to solve the 8/8 task using stereotyped strategies. To investigate this consecutive visits to adjacent arms were computed. A high number of visits to adjacent arms might show a preference for turning in one direction or the implementation of a repetitive foraging pattern. This would suggest that rats may be using a rigid strategy that would not depend on the use of spatial cues. However, in both sets of probe tests the consecutive number of visits to adjacent arms remained low and did not change when compared to performance levels in the original training environment.

The combined results of the first and second set of probe trials suggest that once rats know very well the environment needed to solve a task, smaller subsets of the original environment are needed to effectively navigate and solve a task (at least one that requires food foraging). Therefore, repeated experience renders the minimal necessary spatial map to an ever more skeletal representation where fewer of the original attributes of the environment are needed for navigation as time progresses. This explains why the removal of salient cues or a change in the room's shape did not affect navigation. It seems that starting location with respect to one familiar cue, together with familiar visual and other stimuli are sufficient. Because animals know the environment so well, it seems that they can rely on the use of less prominent cues once an environment undergoes some major change.

The increase in WM errors after the introduction of new cues to the original environment is similar to the increase in errors observed when rats were moved to a new room. Subtraction of information or changes in the information already known do not seem to affect hippocampal functioning. Space can change substantially, still animals can solve a

task as long as new stimuli are not brought. However, the addition of new information, either in the form of new cues or a totally new environment affects navigation. Navigation seems to be affected because the environment essentially is not the same since novel stimuli have to be encoded. New stimuli seem to engage the hippocampal system in a new learning process. This is consistent with changes in the firing of hippocampal place cells after manipulations to a well known environment. In a recording of hippocampal place cells, Tanila, Shapiro, Gallagher, and Eichenbaum (1997) observed that after exposing animals to variations of the same environment, hippocampal cells encode different sets of relationships among cues that depended on each particular manipulation.

The results also suggest that rats might process spatial information hierarchically in terms of the novelty of the spatial attributes needed to solve the task. Newer elements seem to affect spatial processing more than changes in already known information. This is suggested by the contrasting results of two of the probe tests in the first set of tests: the test in which cues were eliminated (fourth probe trial) and the test in which cues were eliminated and then substituted by new ones (fifth probe trial). Different from the test in which cues were removed, performance was affected when salient cues were removed and then were substituted by new ones. In the latter test, the reintroduction of new cues apparently created an ambiguity that affected considerably the navigational strategy. When cues were removed it seems that secondary non-defined aspects of the environment were used to solve the task. These secondary aspects seemed to be relevant when ambiguity was introduced by scrambling cues that were already known or the shape of the room was changed (first through third probe trials of the first set). However, the introduction of unfamiliar cues affected the ability to solve ambiguity. In this case, these unfamiliar cues were more relevant

than secondary information since the old environment was not sufficient for solving the task. It seems that novelty in the form of new salient cues might impose a heavier burden on hippocampal processing since the animal must learn new information. These results are recapitulated in the sixth probe trial of the first set, where testing well-trained rats in an unfamiliar environment affects their ability to solve a spatial working memory task. Since neither knowledge of the task nor self generated movements were sufficient for solving the task in the unfamiliar environment, knowledge of spatial information seems to be the necessary element for working memory processing.

Evidence for a hierarchical processing of environmental stimuli by the hippocampus might be found in a study by Shapiro, Tanila and Eichenbaum (1997). They recorded hippocampal cells in rats well-trained in a plus maze to receive hypothalamic stimulation. Similar to the study in this chapter, cues were located both in the walls of a room defined by curtains and in the floor of the arms of the testing apparatus. Recordings showed that the firing of cells correlated with distinct sets of cues. Rotations of distal cues and the apparatus in opposite directions with respect to one another showed that some cells responded to the distal cues, others to the cues in the maze, and others kept firing in relation to the actual physical space, meaning that they kept responding to unknown cues either in the curtains, floor, ceiling, or any other part of the room. These unknown cues could be visual as well as auditory or tactile (e.g., an air draft). In general, it was observed that some cells would respond to very small sets of cues or even one cue. This shows that encoding of the environment by hippocampal cells in well-trained rats therefore can be limited to very small aspects of the environment. Finally, after rotations, some of the cells did not keep firing in the same way, either changing their activation pattern or not firing at all. Other cells that did

not show place fields started firing after changes in the environment. This means that the hippocampal circuitry intervened in the processing of new information and encoding of a new set of experiences that could be used for navigation in what could be a qualitatively new environment.

The present study also suggests several specific functions for NMDA receptors. In this study a dose of MK-801 that affects PBP did not affect performance in a familiar room. The results are similar to those presented in Chapter 4, where it was observed that NPC 17742 did not affect spatial working memory in the 8/8 radial arm maze task in a familiar room. However, even when rats had been trained for 6 months, those injected with the antagonist became impaired when tested in an unfamiliar room. After learning several novel environments, the training time required to perform the task approaches nil in well-trained saline injected rats. It appears that rats develop a learning set for performing the radial maze task. The antagonist, however, impairs the already learned task when rats have to perform in a new room. The results from this and the previous chapter suggest that NMDA receptors are not necessary for learning the spatial working memory task, nor for performing it. Since blockade of NMDA receptors results in an increase in WM errors when rats are tested in novel environments, it seems that the receptors are necessary only for the encoding of new environmental information. This is consistent with place field studies that show that MK-801 administered before the introduction of rats to a new environment disrupts place field stabilization (Austin, Fortin & Shapiro, 1990).

The results presented in this chapter might have implications for a spatial mapping model. The hippocampus seems to be necessary not so much for creating a spatial map, but for creating a record of experiences related to specific areas of an environment. As

knowledge about an environment grows, only a few aspects of this environment can be relevant at a certain moment for solving a task effectively. A relationship among all or a large set of stimuli is not necessary for solving the task. The hippocampus might allow to process environmental information in a flexible way, segmenting it into smaller sets of information. This would be consistent with an evolutionary point of view. The progressive establishment of multiple relationships among stimuli, instead of the existence of one large rigid map, might allow to solve tasks in ever efficient manner in the face of constantly changing conditions.

Table 5.1. Sets of probe tests done to assess performance of well-trained rats in an eight arm radial maze. Two sets are described. The first set of tests, described on panel A, altered the testing room *before* the rats were exposed to that environment. The second set of tests, described on panel B, altered the testing room *after* the rats were exposed to the maze and allowed to choose four arms.

Table 5.1

A

First set of probe tests
1. Distal cues were scrambled to create a new configuration.
2. Original cue configuration was kept, but the curtains formed a circular room.
3. New configuration of cues was put into the circular room.
4. Distal cues were removed.
5. New cues (a white paper stripe) replaced in the old ones.
6. Rats were tested in an unfamiliar room.

B

Second set of probe tests
1. Rats were given four choices and removed from the maze for 10 min. During the delay the distal cue configuration was altered. The rats were returned to the maze after the delay.
2. Rats were given four choices, were removed from the maze for 1 h, and returned to the maze.
3. Rats were given 4 choices and were removed from the maze for 1 h. The constellation of distal stimuli was rotated 90 ° CCW, and the rat was returned to the maze from an entrance 90 ° CCW from the standard.

Figure 5.1. Performance of normal rats in the 8/8 WM task in the radial arm maze. Each bar represents mean WM errors of all rats. Performance was affected by the introduction of new cues and by the re-testing of animals in a new room with different cues. Performance was not effected by neither cue scrambling nor removal. Changes in room geometry (from square to circular) including or not cue scrambling did not have an effect either.

Figure 5.1

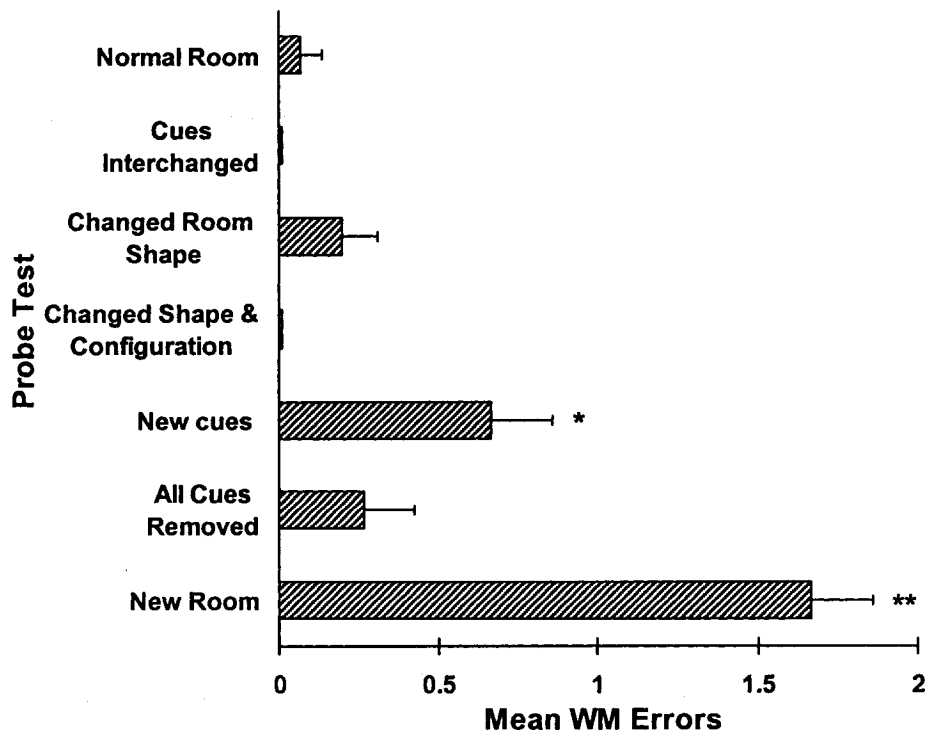

Normal Room
Cues Interchanged
Changed Room Shape
Changed Shape & Configuration
New cues
All Cues Removed
New Room
*
**
Probe Test
0
0.5
1
1.5
2
Mean WM Errors

Figure 5.2. Performance of normal rats in the 8/8 WM task in the radial arm maze with a delay between the fourth and fifth choices. Each bar represents mean WM errors of all rats. Performance was affected by the rotation of cues and re-introduction of animal to the environment through a new entry 1 hr after the fourth choice. The 1 hr delay alone did not produce a deficit, nor did the scrambling of the cues between the fourth and fifth choices.

Figure 5.2

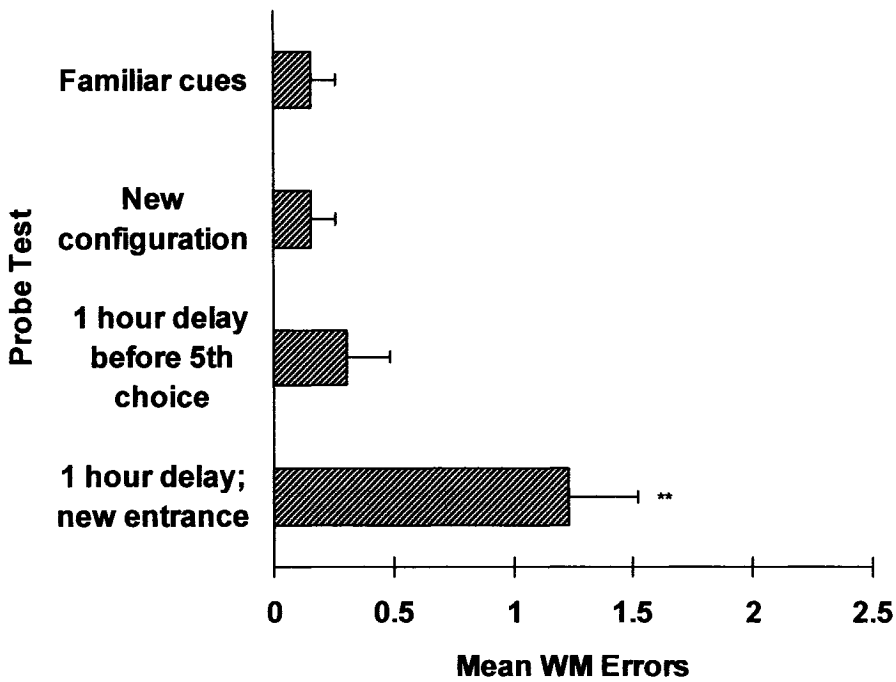

Familiar cues
New configuration
1 hour delay before 5th choice
1 hour delay; new entrance
**
Probe Test
0
0.5
1
1.5
2
2.5
Mean WM Errors

Figure 5.3. Graphs show the number of consecutive visits that rats made to adjacent arms in both sets of probe trials. For each graph, each bar represents mean WM errors of all rats. Panel A shows that environmental manipulations performed before each probe test did not have an effect in rats' visits to arms. Panel B shows that environmental manipulations performed after a delayed fifth choice did not have an effect either. In both cases, the number of arm visits remained closed to baseline levels in the familiar training environment.

Figure 5.3

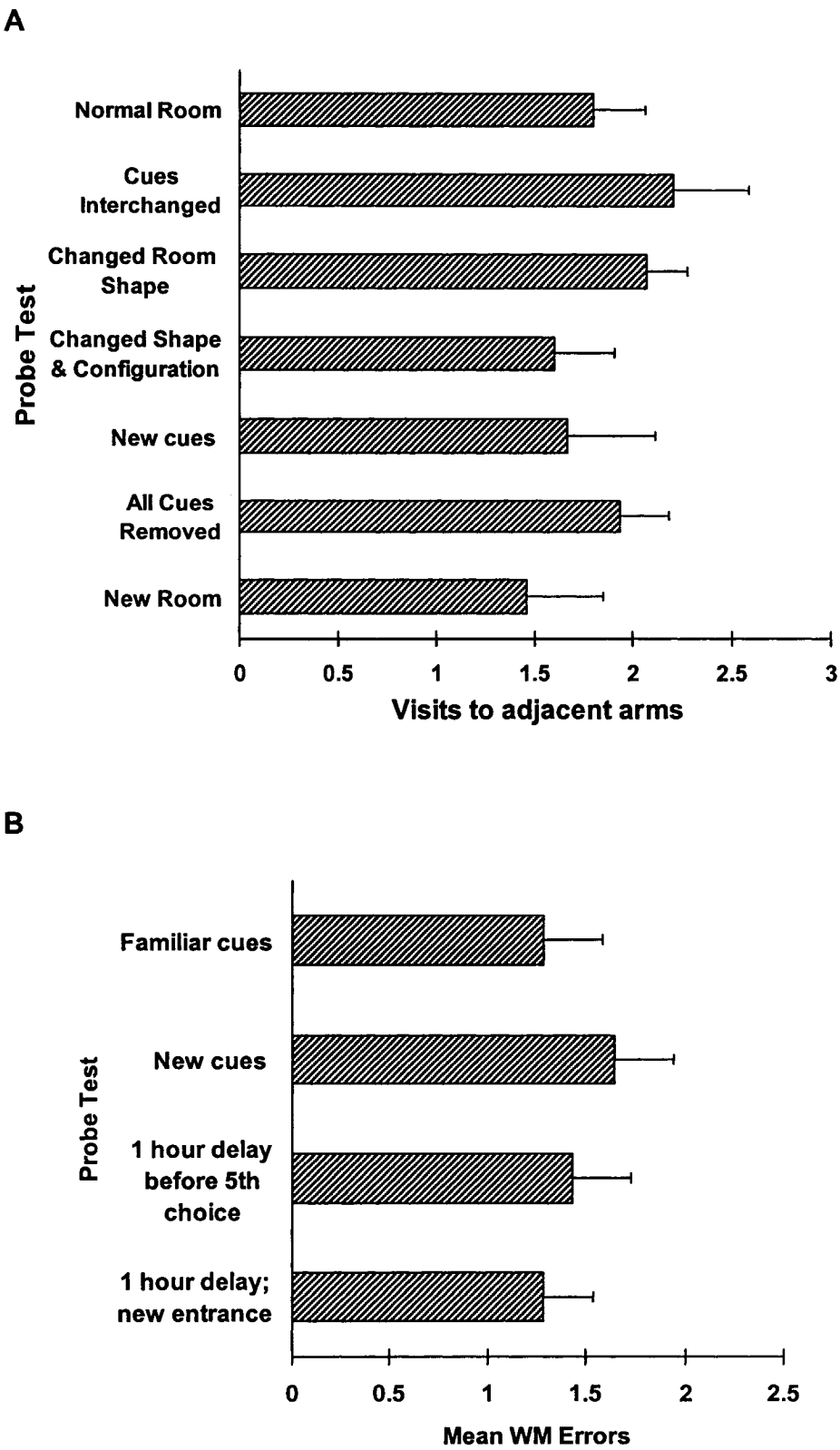
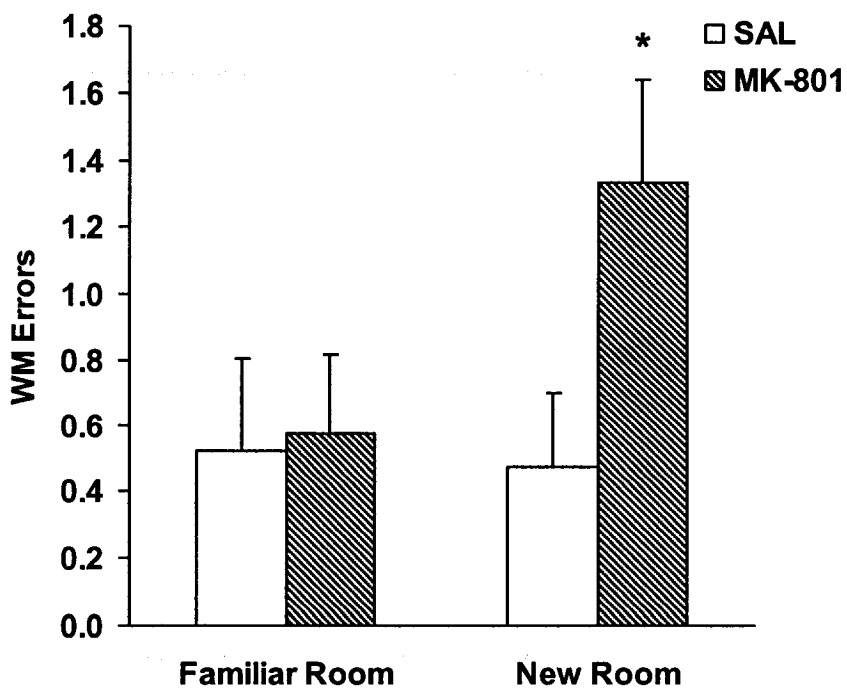

A
Normal Room
Cues Interchanged
Changed Room Shape
Changed Shape & Configuration
New cues
All Cues Removed
New Room
Probe Test
0
0.5
1
1.5
2
2.5
3
Visits to adjacent arms
B
Familiar cues
New cues
1 hour delay before 5th choice
1 hour delay; new entrance
Probe Test
0
0.5
1
1.5
2
2.5
Mean WM Errors

Figure 5.4. MK-801 treated rats performance in the 8/8 WM radial arm maze task did not differ from saline treated rats in a familiar environment where they had been extensively trained for over six months. However, when transferred to an unfamiliar environment MK-801 treated rats made more errors than saline treated rats. After their extensive training in the WM task in the familiar environment all rats showed considerable savings in the new environment, having substantially fewer errors than at the beginning of training. Bars represent the mean for total of WM errors made by rats per trial on a block of three days.

Figure 5.4


1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WM Errors
*
SAL
MK-801
Familiar Room
New Room

CHAPTER 6. GENERAL DISCUSSION

To examine the idea that NMDA receptor-mediated synaptic plasticity is necessary for learning the spatial attributes of an environment, rats treated with NMDA antagonists were tested on a series of electrophysiological and behavioral experiments. The administration of NMDA antagonists NPC 17742 and MK-801 impaired PBP in the perforant path-dentate gyrus pathway in a dose-dependent manner. These results correlated with those of behavioral studies. NPC 17742 impaired a) spatial but not cued learning in the water maze in a dose-dependent manner, and b) spatial learning but not spatial working memory in the radial arm maze. A dose of MK-801 that impaired PBP did not affect the performance of rats that had been extensively trained in the radial arm maze, nor did structured manipulations of familiar cues and room shape. The introduction of new cues impaired performance in untreated rats. MK-801 impaired new learning after rats were exposed to a third novel room.

The results are consistent with the hypothesis that NMDA receptors mediate the learning and organization of new environmental information, but are not relevant for working memory or for performance once a representation of the environment has been established. Radial arm maze results suggest that with repeated experience representations can be activated by smaller subsets of environmental stimuli and used to solve a task independently of other subsets. Once the representation is stable, neither the blocking of NMDA receptors nor changes in already known information impair performance. The fact that performance is disturbed if unfamiliar information (such as novel cues, a new room, or a novel entrance to the testing environment) is introduced, suggests that the representation has to be rebuilt to incorporate this novel information. NMDA antagonists impair this new

learning showing that a high level of pre-training is not sufficient to solve the task efficiently. NMDA receptors are necessary again for this new learning regardless of how well the procedures required to solve the task are known. The convergence of the results of electrophysiological and behavioral studies support the role of PBP as a useful tool for the study of NMDA receptor-mediated synaptic changes that occur during hippocampal-dependent learning.

Potentiation in the hippocampus and the study of memory formation

Among the strategies to study the relationship between LTP and learning (see Martin et al., 2000), pharmacological interventions have been quite succesful (see Steele & Morris, 1999, for a comparison with genetic manipulations and some of its caveats). If a drug that affects the neural mechanisms that mediate LTP also affects learning in a similar and proportionate manner, then this would suggest that both phenomena may be subserved by the same mechanisms. Morris et al. (1986) originally explored this relationship by blocking LTP in the dentate gyrus and impairing spatial but not cued learning in the water maze after intracerebral administrations of AP5. The study supported the notion that hippocampal NMDA receptors are specialized for spatial but not procedural learning, suggesting that qualitatively different forms of learning might be mediated by distinct cellular mechanisms.

Despite the success of pharmacological interventions it is difficult to definitely assert that the mechanisms that support PBP or LTP *are* the same that subserve learning. In the experiments discussed here, NMDA antagonists may have impaired learning and PBP through different mechanisms. An example of this can be found in the study of "behavioral LTP". Sharp, McNaughton and Barnes (1989) found that short-term changes in the dentate gyrus EPs occur after exploratory behaviors. Later, Moser, Mathiesen and Andersen (1993) found that these changes were caused by an increase in temperature due to the animals'

activity and were not related to exploratory behavior. Before an argument for causation can be made, more research is needed. One strategy in this direction would be to use the pharmacological approach to correlate the known phases of LTP (i.e., induction, expression, and maintenance) with pharmacologically-induced disruptions in learning and retrieval. This strategy would contribute to a better understanding of the plasticity mechanisms that may be related to specific stages of the memory formation process.

The fact that pharmacological manipulations that affect NMDA receptors, including those in the hippocampus, generally do not cause deficits in already learned declarative information suggests that NMDA-mediated synaptic processes in the hippocampal system might subserve encoding or consolidation rather than a storage function (see Zola-Morgan & Squire, 1990, for a study on the time limits of the effects of hippocampal lesions on previously learned information). While hippocampal involvement in learning is relatively short-term, LTP is long-lasting, probably at least five weeks (Stäubli & Lynch, 1987). This raises the question of whether such a long-term change can explain the relatively short-term hippocampal mediation of memory.

It is possible that declarative learning involves a temporary change in hippocampal synaptic plasticity that would be a necessary or prerequisite condition for longer-term forms of synaptic plasticity. Since lesions to the hippocampus produce deficits in spatial learning, but not of retrieval in rats and monkeys (see Chapter 1 for a discussion), the hippocampus may support a form of plasticity that influences the long-term storage of information in other parts of the brain, possibly neocortex (see Eichenbaum, 2001b for a discussion of evidence from imaging studies).

Buzsáki (1989) proposed that the hippocampal synaptic plasticity required for memory formation and triggered by exploratory behaviors might involve a short-term form of synaptic plasticity in the dentate gyrus granule cells, followed by longer forms of synaptic strengthening in CA1 and CA3, allowing memory storage in neocortical areas. There is some evidence supporting the transient nature of dentate gyrus morphological changes after spatial learning. The number of synapses in the granule cell layer increases 9 hr after training on a spatial version of the water maze task, but returns to baseline (i.e., pre-training) levels after 24 hr (Eyre, Richter-Levin, Avital, & Stewart, 2003). Others have found similar results, observing an increase in the number of dendritic spines in the mid-molecular layer of the dentate gyrus 6 hr after training rats in the water maze and passive avoidance tasks, with a return to baseline levels 72 hr after training (O'Malley, O'Connell, Murphy, & Regan, 2000; O'Malley, O'Connell, & Regan, 1998). In general, this evidence correlates with the temporary nature of PBP. Therefore, the study of PBP might introduce another perspective in the study of hippocampal synaptic plasticity.

Even if the duration of potentiation observed in PBP and LTP differs, they share some relevant physiological characteristics. Although PBP represents a temporary form of synaptic plasticity, while LTP has been observed for much longer periods, according to Diamond et al. (1988) they have common induction mechanisms. Diamond and colleagues observed that the synaptic changes caused by a PBS applied after LTP had been induced in the same pathway (CA1) were very small. Similar results were obtained when the reverse pattern of stimulation was used (i.e, when LTP stimulation was applied *after* PBP had been induced). The reduction in the potentiation obtained in the second treatment of each case suggests that the pathway had endured some form of saturation. If LTP and PBP induction

involved different mechanisms, then saturation would not be observed and the treatment administered first would not change the effect of the treatment administered second. Therefore, PBP may be subserved by the same mechanisms through which LTP is established, except that it represents a temporary form of plasticity.

The existence of several forms of synaptic plasticity of various durations suggests that memory formation might entail processes that vary in both duration and physiological mechanisms. Kandel and colleagues (Bailey, Giustetto, Zhu, Chen, & Kandel, 2000) have studied five such processes in the marine slug *Aplysia californica*. Some of these last only a few minutes and are characterized by an increase in calcium influx to neurons matched with a decrease in potassium influx and a temporary increase in serotonin release (Boyle, Klein, Smith, & Kandel, 1984; Byrne & Kandel, 1996). Other forms of strengthening are intermediate-termed and might be necessary for long-term strengthening. Intermediate-term sensitization lasts up to a few hours and requires protein synthesis (Ghirardi, Montarolo, & Kandel, 1995). Finally, long-term strengthening involves both protein and mRNA synthesis, eventually leading to structural changes (Steward & Worley, 2002).

Mechanisms similar to the ones observed in *Aplysia* mediate long-term synaptic change in the mammalian brain through the activation of second-messenger pathways (Kandel, 2001). The initial processes that occur after tetanic potentiation are known as early LTP, while the transcription processes that occur later and that involve structural changes are known as late LTP (Bolshakov, Golan, Kandel, & Siegelbaum, 1997; Nguyen, Abel, & Kandel, 1994). Pharmacological and genetic disruptions of transcription prevent the formation of late LTP (e.g., Abel et al., 1997; Frey, Krug, Reymann, & Matthies, 1988; Huang, Nguyen, Abel, & Kandel, 1996). The events that lead to synaptic strengthening are

of different durations and can not be classified simply into two discrete categories such as temporary and permanent (see Kandel 2001). The fact that interventions that affect some of the stages of LTP described above also affect learning (e.g., Abel et al., 1997; Barad, Bourtchouladze, Winder, Golan, & Kandel, 1998), suggests that memory formation should be understood as a continuum.

Although much knowledge has been gained through the study of the induction of potentiation (i.e., early stages), the processes that lead to long-term synaptic strengthening (i.e, late stages) are less undestood. It is known that in general changes in glutamate receptors mediate the expression of potentiation (Malenka & Nicoll, 1999), but there is no agreement on the type, extent, and duration of these changes. In general, studies suggest that modifications occur in both pre- and postynaptic membranes and could include the formation of new synapses, the enhancement of receptors' performance, the unmasking of latent receptors, and the addition of new receptors to existing synapses, which according to Baudry and Lynch (2001) is one of the most plausible mechanisms. In support of this, Voronin and Cherubini (2004) propose that before potentiation some synapses are not active because either there is not enough release of glutamate from the presynaptic neuron or there are no AMPA receptors at the postsynaptic neurons expressing NMDA receptors (however see Renger, Egles, & Liu, 2001). Potentiation would produce an enhanced release of glutamate, inducing the insertion of AMPA receptors in the postsynaptic membrane (see Kullmann, 1994), thus facilitating subsequent normal transmission in the circuit (cf. Collingridge, Kehl, & McLennan, 1983). Whether this is the main mechanism for LTP expression remains to be determined.

Since the 1980s there have been an increasing number of research papers on LTP and associated phenomena (see Malenka, 2003). LTP and PBP in the hippocampal system might serve as tools for understanding the cellular mechanisms that support declarative learning. However, this type of learning and even one of its manifestations, spatial learning, can entail diverse behavioral processes that are mediated by distinct cellular processes. Specific structures of the hippocampal system as well as subregions of the hippocampus proper contribute in unique ways to declarative memory formation (e.g., Gilbert et al., 2001; Lee, Hunsaker, & Kesner, 2005; Lee, Jerman, & Kesner, 2005), while NMDA receptors have various behavioral roles that depend on their location within the hippocampus (see Lee & Kesner, 2002, for an analysis of the behavioral specialization of NMDA receptors located within CA1,CA3, and dentate gyrus). Therefore, the study of the behavioral processes that are included in declarative learning models is a necessary step to understand better any relation between NMDA receptor-mediated potentiation and this type of learning.

NMDA receptors, spatial working memory and spatial learning

Lesions to the fornix (Olton et al., 1979; Olton & Papas, 1979) and dorsal hippocampus (Barnes, 1988; Pothuizen, Zhang, Jongen-Relo, Feldon, & Yee, 2004) cause working memory deficits in the radial arm maze. These studies support the notion that regions of the hippocampal formation are necessary for spatial working memory. However, pharmacological manipulations of the hippocampal system have yielded contrasting results. After the initial suggestion that NMDA receptors are necessary for solving a spatial version of the water maze (Morris et al., 1986), researchers tested this in the radial arm maze using both variations in the training protocol and diverse NMDA antagonists (Butelman, 1989; Caramanos & Shapiro, 1994; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992; Shapiro, Tirado-Santiago, Zayas-Monge, & Zozula, 1996). These studies suggested that

spatial learning but not necessarily spatial working memory depends on NMDA receptor activation. More recent studies have used the water maze to study the role of NMDA receptors in short-term memory (McDonald et al., 2005; Steele & Morris, 1999). Although these studies did not directly test spatial working memory, they measured the role of NMDA receptors in the processing of information that temporarily (i.e, within one training session) is necessary to solve a task.

To compare the effects of hippocampus plus dentate gyrus lesions and the blocking of NMDA receptors in the use of short- and long-term memory, Steele and Morris (1999) studied navigation using a delayed matching-to-place task in the water maze. In this task a hidden platform remains in the same location across trials during a session, but changes to a new location for each session. An intertrial interval of either 15 s, 20 min, or 2 hr is imposed between the first and second trial. All rats were trained for several days without treatment until they reached asymptotic performance, and were then treated and re-trained. While normal rats showed a reduction in latency after the intertrial interval, rats with hippocampus-dentate gyrus lesions did not show any improvement. Rats treated with D-AP5 showed reduced latencies after 15 s delays, but not after longer delays. The delay-independent impairment of lesioned rats can be understood as disruptions in both consolidation and short-term memory, while the delay-dependent impairment observed in D-AP5 treated rats can be interpreted as a deficit in consolidation rather than in short-term memory. D-AP5 treated rats performed well at short delays because remembering the location of the platform in the previous trial would not require NMDA receptor-mediated synaptic plasticity. However, at longer intertrial intervals rats had to consolidate new information about the location of the platform for that day. Thus, D-AP5 presumably affected the synaptic processes that mediate

new place learning. McDonald and colleagues (2005) obtained similar results to those of Steele and Morris (1999) testing CPP treated rats in another variation of the water maze task. After rats had been trained in a standard spatial version of the water maze, they were treated with CPP or saline and re-trained in the same environment to find the platform in a novel location. Both groups showed similar progressive reductions in escape latency across trials. However, they differed in a treatment-free probe trial given after re-training. Rats previously treated with saline initially searched for the platform in the novel location while rats previously treated with CPP searched the platform in the original location. The results suggest that in a familiar environment CPP does not block the remembering of a specific place for short-periods of time, but that it impairs the consolidation of this new information into long-term memory.

The radial-arm maze studies reported in Chapters 4 and 5 measured spatial learning and spatial working memory in separate experiments. To dissociate these behaviors, spatial learning was studied in naïve rats, while working memory was tested in the same environment where rats had already learned the task. Rats were treated with the same dose of NPC 17742 that impaired PBP and that also produced deficits in spatial but not cued learning in the water maze in Chapters 2 and 3, respectively. Because performance was not impaired in the cued task of the water maze, the drug was not expected to cause sensorimotor impairments. Consistent with the water maze study, NPC 17742 impaired spatial learning. Similarly, in Chapter 5, a dose of MK-801 that impaired PBP also impaired spatial learning.

In the radial maze experiments neither drug caused spatial working memory impairments in familiar environments. The introduction of delays of various lengths between the fourth and fifth choices did not have an effect either. It is possible that longer

delays would have caused impairments. However, deficits produced by long delays would be more representative of a consolidation rather than a working memory impairment (see Steele & Morris, 1999). Results show that NMDA receptors are not necessary for working memory in the radial arm maze task. This contrasts with studies where hippocampal lesioned rats are impaired after a delay. Therefore, while the hippocampus is important for processing both spatial cues and information that changes within trials, remembering this information does not depend on NMDA receptor activation. The results of the radial maze studies suggest that once an environment is known, the implementation of a navigational strategy using familiar spatial cues does not seem to depend on NMDA receptors.

Together the results support the idea that within the hippocampal system NMDA receptors are necessary for the consolidation of information into a long-term representation, but are not necessary for processing or encoding information that is necessary for a short period of time. Converging evidence from the electrophysiological and behavioral studies presented here suggests that NMDA receptor-mediated synaptic changes such as those that occur in PBP might account for the building of spatial representations. Once it becomes stable, the use of such a representation would depend on other processes not mediated by NMDA receptor activation. Research that focuses on the molecular aspects of PBP and their correlation with behavioral studies might elucidate the relationship between the synaptic changes observed after PBS and spatial learning.

The hippocampal system and the encoding of experience: Implications for the concept of a spatial map

The experiments in Chapters 4 and 5 show that once a spatial task is learned to asymptotic levels, spatial navigation is not affected substantially either by the blockade of NMDA receptors, structured manipulations of the familiar training environment, or the

addition of long within-trial delays. However, once novel information is introduced to the familiar environment, performance is impaired because this information imposes a burden on neural processing by promoting changes in synaptic plasticity. These changes are presumably mediated by NMDA receptors since the original level of proficiency attained in the radial maze (i.e, near errorless performance after 6 months of training) was not sufficient to supersede the effects of NMDA antagonists in the learning of a novel environment. The deficits caused by the drug can not be attributed to sensorimotor disturbances since performance was not affected by the drug when given to rats pre-trained in the familiar environment.

The fact that multiple changes in the testing environment (with the exception of those that introduced new cues) did not have an effect on performance supports the idea that learning about space does not necessarily correspond to the recording of a topographical or geometrical map of the environment. Shapiro and Eichenbaum (1999) redefine the idea of a spatial map and call it a "memory map" in which "hippocampal cells encode an ongoing record of regularities, consistencies, and novelties that comprise the unfolding structure of episodes." (p. 371). Evidence from place cell studies shows that there is no consistency in the way any particular population of cells responds: while some cells constantly fire in relation to space, other cells respond to stimuli regardless of their position in space, and their firing does not necessarily correspond to the geometrical characteristics of space (see Wood, Dudchenko, & Eichenbaum, 1999, for a study place cell study using odor recognition).

The results of Chapter 5 suggest that as learning progresses, the environment is encoded by multiple and smaller subsets of stimuli. As a familiar environment undergoes changes, one or few of these subsets can activate the original representation of the

environment. If the neural representation of memory is understood as patterns of activation of a circuit composed of multiple units (i.e, groups of cells that fire in coordination), the particular experiences that an organism has in an environment might be encoded by a specific and maybe unique pattern of activity in those units. These units can encode reference points that are autonomous and efficient navigation might depend on a small subset from a larger representation whenever changes in the environment occur. This might explain why the removal of cues in Chapter 5 did not affect overall navigational activity of well-trained animals. Some static or undefined cues may have served as points of reference. Spatial navigation therefore would not depend on one giant topographical map, but on multiple patterns of neural activity that can interact or act separately contingent to changes in the environment.

Evidence for the existence of multiple patterns of neural activity that guide navigation can be found in place cells studies. Some of these studies have shown that when changes are made in a familiar environment, cells respond independently to specific subsets of cues (e.g., Shapiro, Tanila, & Eichenbaum, 1997; Tanila, Sipila, Shapiro, & Eichenbaum, 1997). This would suggest a mechanism at the cellular level for the encoding of experience as sets of episodes. Shapiro et al. (1997) recorded place cells in rats on a four-arm radial maze located in a cued controlled environment very similar to the one described in Chapter 5. After place cells were identified, local and distal cues were moved. One of the manipulations consisted of the rotation of distal and local cues in opposite directions by 90 degrees, creating the effect of a 180 degrees rotation of some cues in relation to others. After the rotations, place cells responded to combinations of local and distal cues. Repetitions of the double rotations eventually changed the firing of some cells, while leaving others

unchanged. It seems that exposure to a new environment (i.e., the double rotated cues) caused some cells to encode a new set of experiences in the environment while leaving others responding to the original configuration. Therefore, far from one environmental representation being replaced by another, both eventually became encoded. This would allow the animals to efficiently navigate two similar environments by identifying each ones' particularities.

The results of Shapiro et al. (1997) and those presented in Chapter 5 are congruent. In the studies reported here, rats' performance was resilient to environmental changes suggesting that efficient navigation was not guided by one unitary geometric representation. As long as rats were not exposed to novel cues or a new room, after each manipulation navigation could have been guided by small subsets of the environment that allowed efficient performance of the 8/8 task. The learned representation was used flexibly.

Eichenbaum and colleagues (Eichenbaum, 1996, 2000, 2001a; Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999; Shapiro & Eichenbaum, 1999) propose that the hippocampus mediates the processing of information in a flexible way. According to Eichenbaum (1996) the traditional concept of a cognitive map can be understood as the integration of information of different sorts in memory and the flexible use of information in novel situations (p 190). Thus, a representation would include not only information about the environment but also strategies for using environmental information. One example of this can be found in a study by Eichenbaum, Stewart and Morris (1990) in which fornix lesioned rats were able to learn a spatial learning task in a water maze only if they were started from the same location for every trial. However, rats were impaired if they were placed in different starting locations for each trial or if cues were rotated in relation to the

starting location. Therefore, fornix lesioned rats learned to find a place using a stereotypical response that was guided by distal cues, but were not able to use the spatial representation in conditions that required implementing different navigational paths. The deficit observed in fornix lesioned rats is not related to the type of material they had to learn, but to the computational demands upon the hippocampal system. Apparently, as long as a spatial task does not require flexibility, hippocampal lesioned rats can solve it. The hippocampal system thus might be required for learning different episodes and relating the commonalities among these to implement a general rule in novel situations, while at the same time identifying unique aspects of each episode.

The anatomical and physiological organization of the hippocampus and related structures might explain how the hippocampal system processes information about the environment in this flexible way. Eichenbaum (2004) discusses convergent afferents, recurrent connections, and LTP as three properties of the hippocampal system represented in area CA3 that allow three interrelated processes involved in behavioral flexibility: associative representation, sequential organization, and relational networking. The hippocampus receives convergent afferents from all cortical association areas. According to the model, once information from these areas simultaneously reaches the hippocampus, it is associated in cells of area CA3 through recurrent connections which are mostly excitatory and glutamatergic. The synapses of these connections can endure fast changes in synaptic plasticity such as LTP. During a normal learning experience, NMDA receptor-mediated synaptic changes would occur in the hippocampus as information from cortical areas converges simultaneously in the structure. Other information that reaches the hippocampus in sequences activates cells in a sequential manner, probably establishing asymmetrical

connections in CA3. According to the model, an episode would be encoded in cells that fire in sequence. If partial information activates the circuit, the rest of the sequence that encodes the episode is activated. Finally, the circuit would also represent similar discontiguous episodes as similar patterns of activation, establishing a relational network. Information that is particular to one episode is represented in the network as a different pattern of activation that allows the episodes to be distinguished. The circuit therefore would be able to establish differences and similarities at the same time.

The flexibility of the computations carried out by the hippocampal system can be observed in the ability of rats (and humans) to establish relations between events belonging to different episodes. Any episode can be disassembled into discrete events that can be organized chronologically into a narrative that recreates the essential characteristics of an episode. At the same time, discrete events can be associated through commonalities in ways that are not sequential and that do not correspond to one specific episode. The inability of rats with hippocampal damage of making transitive inferences suggests that the structure is necessary for establishing associations between discontiguous events (Dusek & Eichenbaum, 1997). Events belonging to different episodes are related in a relational network because of their commonalities. With time, these relational networks become decontextualized from the specific moment in which they occurred and give way to semantic or generalized knowledge about the specific properties of objects.

In general the hippocampal system allows mammals to actively acquire information and relate it to previous experiences in flexible ways through connections with the neocortex and parahippocampal cortex (see Eichenbaum, 2000). The flexibility of this organization would consist in the relation that the system would do of similar aspects shared by new

incoming and already stored information. Incoming information would therefore change the representation of already stored memories, through the establishment of new networks of associations or a refinement of already existing networks to accommodate new material. The organization of novel information is given through NMDA receptor-mediated synaptic plasticity. This plasticity mechanism could resemble a process similar to the changes observed during LTP and PBP.

Conclusion

Both PBP in the dentate gyrus and spatial learning depend on the activation of NMDA receptors. The activation of these receptors is necessary for learning stable representations of the environment, but is not relevant for processing information that is used only for short periods of time such as in spatial working memory tasks. Blocking of NMDA receptors disrupt the building of new representations of the environment regardless of the familiarity with the procedural demands of the task.

In general, spatial learning entails an internalization of the content as well as the organization of environmental stimuli. As learning proceeds, the internal representation can be activated by smaller subsets of the original environmental cues. The representations therefore are encoded as relationships of stimuli that share regularities or that are unique to a particular episode. The relations among stimuli presumably are encoded by inputs to hippocampal neurons from the cortex, and by recurrent connections within the hippocampus, both of which require NMDA receptor dependent plasticity.

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APPENDIX A

APPENDIX A. Research on the human hippocampus and its relevance for the understanding of the learning and memory deficits in neuropsychiatric conditions

A. Relevance of the study of learning and hippocampal functioning: Applied and theoretical considerations

Clinical research has presented consistent evidence of the role of the hippocampal system in neurological disorders that involve learning deficits. The hippocampus suffers neuronal loss in epilepsy (Dam, 1980; Quigg, Bertram, Jackson, & Laws, 1997). The hippocampal formation also suffers massive degeneration in some devastating neuropsychological dementing disorders such as Alzheimer's disease (Ball et al., 1985; Hyman, Van Hoesen, & Damasio, 1987, 1990; Hyman, Van Hoesen, Damasio, & Barnes, 1984), herpes simplex encephalitis (Kapur et al., 1994; Yoneda, Mori, Yamashita, & Yamadori, 1994), and AIDS related dementia (Chang, 1995; Fox, Alford, Achim, Mallory, & Masliah, 1997; Grant et al., 1987; Reyes, Mohar, Mallory, Miller, & Masliah, 1994). Hippocampal degeneration, atrophy, receptor down regulation, or altered neurochemistry have also been associated with neuropsychiatric disorders such as depression (Dubrovsky, 1993; Krishnan et al, 1991; Sheline, Sanghavi, Mintun, & Gado, 1999; Van Elst, Eber, & Trimble, 2000), stress (McEwen, 1999; McEwen & Magarinos, 1997), bipolar disorder (Senitz, 1999; Vawter, Howard, Hyde, Kleinman, & Freed, 1999), stress induced dissociative amnesia related to the so-called false memory syndrome (Bremner, Krystal, Charney, & Southwick, 1996), Cushing's syndrome (Forget, Lacroix, Somma, & Cohen, 2000; Starkman, Gebarski, Berent, & Schteingart, 1992; Stokes, 1995), post-traumatic stress disorder (Bremner, 1999; Bremner, Krystal, Southwick, & Charney, 1995; Sapolsky, 2000; Yehuda, 1999), post-traumatic stress disorder associated with childhood sexual abuse

(Bremner et al., 1999) and, more controversially, with schizophrenia (Benes, 1999; Jonsson, Luts, Guldberg-Kjaer, & Ohman, 1999; Zaidel, Esiri, & Harrison, 1997; see Nelson, Saykin, Flashman, & Riordan, 1998, for a meta-analysis of the literature). Finally, the hippocampus is the brain structure most affected in transient global amnesia which is characterized by temporary amnesic episodes that may last for several hours (Fogelholm, Kivalo, & Bergstrom, 1975; Frederiks, 1993). Although of heterogeneous etiology and symptomatology, a common denominator of all these illnesses is the presence of some degree of anterograde memory disruption.

At the theoretical level, the study of the hippocampal system is an integral part of the study of the biological basis of learning and memory. Learning and memory have been prevalent topics in the history of psychology. Many of the early schools of psychology had at their core a model or theory of learning and memory. The search for the neural basis of learning and memory however was not one of the main aims of many of these models. About half a century would pass until the first advaces in this regard were made. When research started to gather, it showed a special role of the temporal lobes and, later, of the hippocampal formation in learning and memory functions (Squire, 1987).

The study of changes in behavior due to damage of the hippocampal formation in humans and other mammals progressively led to a rethinking of some behavioral models about learning and memory that originally were not concerned with a neurological functions. Psychological models about learning and memory not only started to explore brain mechanisms, suffered a major restructuring in the way learning and memory are conceptualized. Two of the most important contributions of this intellectual development

have been the understanding that a) learning is not a unitary function, and b) learning and memory are related but dissociable process at the brain level.

Several models about the role of the hippocampus in learning and memory have arisen, identifying the structure as important for the processing of information of different sorts: cognitive, contextual, configural, spatial, explicit, declarative, episodic, relational, and flexible processing. Models that propose that the hippocampus is involved in these processes are not necessarily contradictory (and indeed usually they are not), but refer to particular ways in which researchers operationalize what is meant with processing. Sometimes, a process might be seen as a major categorical umbrella that encompasses other processes, as can be observed in the relationship between declarative and episodic. Spatial processing is seen as one of various dimensions of declarative and episodic processing. Here I will focus on the processing of space and it's relationships with other levels of processing as exposed by various researchers.

As discussd in Chapter 1, early studies of hippocampal function in humans consisted of reports of single case studies of patients with hippocampal lesions. As knowledge about the role of the hippocampus in human learning and memory advanced, groups of patients that shared similar characteristics were studied. Later, the study of patients suffering from neurodegenerative disorders provided a means to study the effects of hippocampal damage on cognitive functions a) over the course of time and b) an on many subjects that shared characteristics in terms of etiology and symptomatology. More recently, advances in technology such as computer assisted imaging techniques, permited the study of the intact hippocampus. These avenues of research have contributed uniquely to understand better hippocampal functioning. This knowledge contributes both to a better understanding of

human cognitive functioning and the development of better treatments for neurodegenerative conditions that affect the hippocampus.

B. Approaches for the study of human hippocampal functioning

1. The broken system: Hippocampal damage and learning in neurodegenerative disorders

Originally Alzheimer's disease was considered a cortical dementia, but later it was recognized as a disorder of limbic degeneration (Hopper & Vogel, 1976). Most Alzheimer's patients suffer from entorhinal cortex damage and virtually all suffer from hippocampal damage (Ball et al., 1984; Hyman, Van Hoesen, Damasio, & Barnes, 1984). Since widespread neurodegeneration is not uncommon during the disease, the study of patients on an early stage allows to understand better the relation between symptomatology and hippocampal degeneration (see Nagy et al., 1999). Hippocampal atrophy seems to be a constant feature in the very early stages, as measured by computed tomograhy (Nagy et al., 1999) and magnetic resonance imaging (Fox et al., 1996). Another common sign early in the disease is the degeneration of the entorhinal cortex. Even in mild cases of the disease there is a reduction of approximately a third of the neurons of the whole entorhinal cortex, but of about 60 % in layer II (Gómez-Isla et al., 1996; Juottonen et al., 1998). In severe cases of the disease the neuronal loss in layer II amounts to 90 %. The decline in hippocampal mediated learning is closely related to this degeneration since layer II of the entorhinal cortex represents the main input to the hippocampal formation via the perforant path.

During the disease, other structures related to the hippocampus suffer degeneration. For example, the dentate gyrus shows a marked reduction of synaptic density and an increase in synaptic apposition, specially but not restricted to the molecular layer (Scheff, Sparks, &

Price, 1996). The subiculum is also implicated. In an uncommon observation (see Ball et al., 1984), using magnetic resonance Mori and colleagues (1997) determined that although there was no hippocampal degeneration in a sample of Alzheimer's disease patients, these showed a marked subicular degeneration which correlated with impairments on a variety of verbal and non-verbal learning tasks. In conclusion, Alzheimer's disease seem to involve degeneration of either the hippocampus or its efferents and afferents.

In general, Alzheimer's patients gradually suffer from learning impairments for facts and events early in the disease (Butters, Delis, & Lucas 1995; Locascio, Growdon, & Corkin, 1995). Similar to patients with hippocampal damage due to ischemia or surgical removal of the structure, they have problems in recognition of visual stimuli, but have no problems in visual priming (Gabrieli, Keane, Stanger, Kjelgaard, Corkin, & Growdon, 1994; Postle, Corkin, & Growdon, 1996). They also show deficits when remembering written narrative information, although they show an improvement in the time required for each subsequent reading of the same passage (Monti, Gabrieli, Wilson, & Reminger, 1994). They also have preserved learning and memory of motor skills even when they do have poor recollection of performing the task before (Gabrieli, Corkin, Mickel, & Growdon, 1993). Together, these data show that Alzheimer's disease patients preserve the learning of some skills.

A caveat in the study of Alzheimer's patients is that eventually, as the disease progresses, patients start to have problems for memories dating periods before the onset of the disease (Sagar, Cohen, Sullivan, Corkin, & Growdon, 1988). This impairment shows a gradient toward ever more remote memories that correlates with the severity of the disease. This might imply damage to cortical areas and would constitute a problem in the study of hippocampal functioning.

To rule out the possibility that other brain structures might be related to cognitive impairments in Alzheimer's disease, studies that correlate imaging of the hippocampus with cognitive decline are helpful in the understanding of human hippocampal functions. For example, Fama and colleagues (1997) correlated both hippocampal and cortical volumes of 50 Alzheimer's patients with measures senstitive to anterograde amnesia using magnetic resonance. They found a strong correlation between hippocampal reduction and low dementia scores, but no correlation between cortical volume and scores. In another study, radiologists blind to the diagnosis of patients with several neurodegenerative disorders that involve learning and memory impairments were asked to assign images obtained using computer tomography and magnetic resonance to a diagnostic category (Horn et al., 1996). The physicians were able to accurately identify the brain images of Alzheimer's patients in 95 % of the cases. The constant neuropathological characteristic of these patients was hippocampal atrophy. Juttonen and colleagues (1998) obtained similar results in a study with mild cases of Alzheimer's disease. Using magnetic resonance imaging they found that the entorhinal cortex allowed for a discriminative accuracy of 92 % between patients with the disease and control subjects.

Longitudinal studies are another strategy for the study of hippocampal function in Alzheimer's disease. Nagy and colleagues (1996) did a longitudinal study with 41 Alzheimer's patients using various measures of learning and other cognitive tasks and later correlated cognitive performance to hippocampal atrophy as measured during autopsy. They found a high correlation between the extent of damage to the hippocampus (as measured by deposition of beta amyloid, neuritic plaques, and neurofibrillary tangles) and low scores on the last measures given to the patients shortly before their deaths. Measures that assessed

learning and memory capacities showed a stronger correlation between hippocampal atrophy and low scores. In another longitudinal study, Fox and colleagues (1996) measured cognitive abilities in asymptomatic subjects at risk of autosomal dominant familial Alzheimer's disease and correlated them to hippocampal volumes obtained using magnetic resonance imaging. Their findings were similar to those of Nagy and colleagues (1996). During the course of three years, among the subjects that started to express the disease, they found a marked reduction of the hippocampus (at a rate of about 8 % per year) which correlated with low scores in both verbal and visual memory. In particular, the approach followed by this last study seems to be a powerful strategy in the study of the functions of the hippocampal system in humans since it allows the assessment of functions before and during the disease. It also allows to study groups as a complement to the study of single case studies.

Human hippocampal function has been studied in epilepsy. Any neurological damage related to this condition develops slowly over a long period of time. Reports of learning impairments in patients with hippocampal damage related to severe epilepsy correlate with the ones of Alzheimer's disease discussed above. For example, in groups of epileptic patients it has been observed that progressive neuronal loss in region CA1 is strongly correlated with the impairment of learning of verbal information (Sass, Buchanan, Kraemer, Westerveld, Kim, & Spencer, 1995). In addition, damage to region CA3 and the hilar area of the dentate gyrus of epileptic patients has been correlated with learning impairments (Sass et al., 1992). Other studies of epileptic patients who have had surgery to various temporal lobe regions show that the most severe learning impairments are associated to the removal of portions of the hippocampus (Miller, Lai, & Muñoz, 1998).

Cushing syndrome is another neurodegenerative condition that involves damage to the hippocampus. During the disease, high levels of glucocorticoids have been associated with hippocampal damage (Sapolsky, Uno, Rebert, & Finch, 1990). Patients diagnosed with Cushing syndrome who have damage to the hippocampus as assessed by magnetic resonance have problems on the recall of verbal material (Starkman, Gebarski, Berent, & Schteingart, 1992). The extent of the deficit correlates with volumes of the hippocampus, subiculum, and dentate gyrus.

The neurological conditions discussed above have as a common pathological marker the degeneration of the hippocampus or its afferent and efferent connections. One of these connections, which has sparked a very early interest in the animal literature is the fornix (e.g., DeVito & White, 1996; Gaffan, 1972, 1974; Mahut, 1972). Similar to patients with hippocampal damage, patients that suffer damage restricted to the fornix have problems acquiring new information, but have no problem remembering information of events that happened before the damage ocurred nor they have problems in the learning of skills (Calabrese, Markowitsch, Harders, Scholz, & Gehlen, 1995; D'Esposito, Verfaellie, Alexander, & Katz, 1995). There seems to be a specialization within the fornix. Heilman & Sypert (1977) argue that damage to the posterior and not the anterior fornix produce severe anterograde amnesia. However, the cases of fornix lesions in humans are not abundant, therefore there is no conclusive evidence on the effects of fornix lesions in humans.

2. The intact system: The study of the role of the intact hippocampus in learning through brain imaging

Another approach to study hippocampal structure and functioning is the use of imaging techniques. Many of the neuropsychological analyses carried until the late 1980's depended on the report of neurosurgeons about the extent of the removal of the tissue done

on epileptic patients. Few studies of amnesic patients used imaging techniques. A 1979 imaging study of hippocampal damage on an amnesic patient by Muramoto and colleagues seems to be the oldest study of this sort (Muramoto, Kuru, Sugishita & Toyokura, 1979). Studies of patients with hippocampal surgeries gave imprecise accounts of the extent of the damage. However, the development of computerized imaging techniques has allowed for a better understanding of the relationship between structure and function in subjects with or without neurological damage (see Press, Amaral, & Squire, 1989, for one of the first protocols developed for imaging the hippocampus with magnetic resonance).

One of the important developments of imaging techniques is the ability to study brain metabolism. This has permited the study of hippocampal functions not only in patients but also in normal subjects. In functional magnetic resonance imaging studies it has been observed that the hippocampus is active during the encoding of novel pictures (Gabrieli, Brewer, & Poldrack, 1998; Stern et al., 1996). Also in positron emission tomography studies, the hippocampus is active not only during the first presentation of geometrical patterns but also during the later recognition of these (Roland & Gulyas, 1995). Other medial temporal lobe structures show a distinctive pattern of activation. For example, the entorhinal cortex is not active upon first presentation of pictures, but it's level of activation in recognition tasks correlates with performance (Fernández, Brewer, Zhao, Glover, & Gabrieli, 1999). Similarly, the level of activation of the parahippocampal gyrus during the encoding of pictures serves as a predictor for the subsequent remembering of the picture (Brewer, Zhao, Desmond, Glover, & Gabrieli, 1998). Together, these studies support the idea that the medial temporal lobe is involved in the learning of declarative information, at

least for pictures, and that there are functionally different areas within this system that are associated with different phases of the learning experience.

Similar results have been observed for the processing of words. There is a very strong correlation between the activity of the left hippocampus during the listening of words and accuracy in the recall of the words 24 hr later, as measured by positron emission tomography (Alkire, Haier, Fallon, & Cahill, 1998). Converging results have been obtained with intracranial event-related potentials (Fernández et al., 1999).

3. The developing system: The study of changes in normal hippocampal function across time through developmental research

Another line of research for the study of human hippocampus role in learning and memory is the assessment of behaviors during development. Cognitive learning and memory change with age (Piaget, 1937/1985). Therefore, the study of the hippocampus and related structures across the life span seems a logical research step. Although it has been suggested that in primates the hippocampal memory system matures more slowly than brain systems related to the learning of habits and skills (Bachevalier & Mishkin, 1984), there is not much evidence for this in humans. This research however has focused in the study of aging.

Aging studies have produced mixed results partly due to the fact that most aged people do not present noticeable declines in cognitive functions (Birren, 1970). Therefore, studies about aging have to concentrate in subpopulations of elders who suffer from various degrees of mild cognitive impairments. Cognitive learning is one of the most affected functions. In a comprehensive study of a retrospective analysis of 1,258 autopsies performed on patients from a geriatric hospital, it was found that there was a strong correlation between age and an increased accumulation of neurofibrillary tangles in area CA1 of the hippocampus

and the inferior temporal cortex (Giannakopoulos, Hof, Mottier, Michel, & Bouras 1994). The accumulation of neurofibrillary tangles was sparse or moderate in other cortical regions, did not increase with age, and was about the same in both non-demented as well as Alzheimer's patients. Other studies however show a correlation between the mild memory loss observed in subpopulations of aged people and hippocampal atrophy. Golomb and colleagues (1993) measured hippocampal volume in 154 normal subjects of 55-88 years of age using either computed tomography or magnetic resonance imaging and correlated hippocampal volume of the patients with their scores in standarized tests that measure memory. The researchers observed that a third of the subjects showed hippocampal atrophy and that this increased with age. This subgroup of people with hippocampal atrophy scored poorly on cognitive measures. Later, in a longitudinal study on which the memory abilities of non-demented aged participants were evaluated for about four years, hippocampal atrophy, as measured by magnetic resonance, was correlated with progressive memory decline (Golomb et al., 1996). On the other hand, many aged people who suffer from mild cognitive impairments do not present neuropathological markers in the hippocampal system (Morrison & Hof, 1997). This might suggest that areas related to cognitive processing other than the hippocampus might get affected during age. Of particular interest is the fact that, on average, older people have more difficulties in recall than in recognition (Craik & McDowd, 1987). Usually, patients with hippocampal damage have equivalent levels of impairment in both functions. Therefore, other factors than hippocampal damage may account for the differential pattern of memory impairments observed in older people.

C. Role of the human hippocampus in spatial learning

1. Spatial learning and the hippocampus in neurodegenerative disorders

The study of Alzheimer's disease patients has shown that the hippocampus is necessary for learning spatial information (Flicker, Bartus, Crook, & Ferris, 1984; Grossi, Becker, Smith, & Trojano, 1993; Henderson, Mack, & Williams, 1989; Trojano, Chiacchio, De Luca, & Grossi, 1994). Since the main neuropathological characteristic of Alzheimer's disease is damage to or diconnection of the hippocampus (Hyman, Van Hoesen, & Damasio, 1987, 1990; Hyman, Van Hoesen, Damasio, & Barnes, 1984; Hyman, Van Hoesen, Kromer, & Damasio, 1986) it seems that the spatial deficits observed in this population are due to hippocampal damage. Although other temporal lobe areas suffer neurodegeneration, it has been found using magnetic resonance imaging that the hippocampus is the structure that shows the most damage during the disease (Jack, Petersen, O'Brien, & Tangalos, 1992). However, it is difficult to determine whether the spatial deficits observed in Alzheimer's patients are due to hippocampal damage alone, since many of these patients also show damage to the parietal cortex (Adelstein, Kesner, & Strassberg, 1992; Giannakopoulos, Gold, Duc, Michel, Hof, & Bouras, 2000). Furthermore, it has been suggested that due to the different patterns of neurodegeneration and impairments seen in Alzheimer's patients a different classificatory system should be used (Binetti, Magni, Padovani, Cappa, Bianchetti, & Trabucchi, 1993). Therefore, with Alzheimer's disease populations, sometimes it is difficult to provide data concerning the involvement of the human hippocampus in spatial abilities.

Evidence from epileptic patients who have undergone surgical removal of the hippocampus might provide more clear cut results about the role of the hippocampus in spatial learning. Some medial temporal lobe epileptic patients who are not receptive to

medication are subject to unilateral hippocampal resection. This subset of epileptic patients allows for a more specific study of spatial memory where lesions are restricted to a particular area, in this case, the hippocampus. For example, it has been observed that patients with right hippocampal removal have severe deficits in spatial learning (Abrahams, Pickering, Polkey, & Morris, 1997; Feigenbaum, Polkey, & Morris, 1996; Nunn, Graydon, Polkey, & Morris, 1999; Nunn, Polkey, & Morris, 1998; Smith & Milner, 1981). Interestingly, patients with left hippocampal removal do not have spatial learning deficits. However, they present problems in verbal learning (Seidenberg, Hermann, Schoenfeld, Davies, Wyler, & Dohan, 1997). Patients with left hippocampal damage are impaired in the recall of words but not pictures (della Rocchetta, 1986). They are also impaired in the recall of non-associated word pairs after a delay is imposed (Rausch, 1987-88). Patients with left-hippocampus lesions also have problems in the learning of narrative material which might reflect an involvement of the left hippocampus not only in lexical learning but also in discursive material such as stories (Frisk & Milner, 1990). In general, it has been observed that there is a high correlation between neuronal loss in the left hippocampus and the extent of the severity of verbal memory impairments (Sass, Westerveld, Buchanan, Spencer, Kim, & Spencer, 1994). This correlates with O'Keefe and Nadel's (1978) suggestion that in humans the left hippocampus may be related to language processing.

2. Spatial learning and the intact human hippocampus

Imaging studies with normal subjects have provided evidence that the hippocampus is involved in spatial learning and memory. Ghaem and colleagues (1997) trained normal subjects to learn a route and later asked them to mentally navigate it during a positron emission tomography scan. While performing the mental navigation, the medial portion of

the hippocampus became active together with the precuneus and insula. In a similar study, using also positron emission tomography, subjects were asked to remember a known place and also to remember a route of how to get to the place including the necessary movements to get there (Berthoz, 1997). In the second task, again the hippocampus together with the precuneus and the insula were active. Together these studies show that the hippocampus had a function in the retrieval of a navigational path.

Other imaging studies have focused on the role of the hippocampus in spatial navigation of well known places. This is different from Ghaem et al. (1997) study where participants had to learn routes during the experiment. In this case it could be said that episodic memory was evaluated. However, in a positron emission tomography study with taxi drivers that had extensive knowledge of London, Maguire, Frackowiak and Frith (1997) assessed semantic topographical memory. They asked participants to describe a well known route traveled by them on a frequent basis. At other times the investigators asked participants to describe easily recognizable landmarks that were not familiar to drivers in terms of spatial navigation (e.g., New York's Statue of Liberty). Functionally speaking, the first task required the establishment of relationships of places and distances while the second was equivalent to remembering a visual image like a picture. For both tasks the parahippocampal gyrus, the posterior cingulate gyrus, the occipitotemporal regions, and the medial parietal area were active. However, the right hippocampus was active during the spatial navigation memory task, but not during the landmarks task. Therefore in this task the right hippocampus seems to be involved not much in remembering a familiar environment as in navigating in a well known place. This type of memory that entails remembering not only

a place, but also navigation strategies to get to the place, has been referred as topokinetic memory (Berthoz, 1997)

The recognition of places as opposed to the navigation of them seems to be related to the parahippocampal gyrus. In functional magnetic resonance imaging, the parahippocampal cortex of normal human subjects shows activity during the static presentation of a room but not of objects (Epstein & Kanwisher, 1998). The levels of activation of this structure did not vary whether the room was empty or furnitured with diverse objects. Moreover, the level of activation of the structure to the rooms was twice as that observed during presentations of pictorial visual representations of the same furniture on a blank background.

Maguire and colleagues did a similar study were positron emission tomography scans were obtained from subjects that explored a virtual reality environment of furnished and non-furnished rooms (Maguire, Frith, Burgess, Donnett, & O'Keefe, 1998). In this study, the right but not the left parahippocampal gyrus was active during the exploration of the furnished but not of the unfurnished rooms. Differences between this and the Epstein and Kanwisher (1998) study might be due to the use of three dimensional vs. two dimensional environments, plus the ability to (virtually) navigate the first instead of just visually examining the second.

Other studies show that the right hippocampus mediates the learning of pictorial information. For example, patients with right but not left hippocampal damage show impairments on the recall of abstract designs after 24 hr of presentation (Jones-Gotman, 1986). Also, lesions to the right hippocampus affect more the recall of pronounced concrete words that are accompanied by its visual representation than the recall of abstracts words presented in the same manner (Jones-Gotman, 1979). It is possible that pictorial information

involves the analysis of several features in relation to each other in terms of position and distance in a manner that could require spatial processing.

APPENDIX B